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GONAD HORMONES AND SEX DIFFERENTIATION[1]

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MY worthy predecessor, Professor W. R. Coe, of Yale University, in his retiring address before Section F in 1939 charmingly paved the approaches to the present discussion. Speaking on "Divergent Pathways in Sexual Development" (Coe, 1940), in his inimitable manner, Professor Coe unfolded for us the devious pathways utilized by invertebrates to attain the same end, *i.e.*, the reproduction of the species. He painted the picture of ingenious mechanisms employed, the manifold deviations from a common pattern, the oscillations from one sexual state to the opposite, and even the dangerous positions of the lowly male when his functions had been fulfilled; the end might be oblivion, a change to the opposite sex, death from natural causes or decapitation by the female in a fit of psychosis.

Just prior to utter exhaustion from the beautifully executed synthesis Professor Coe recalled that there were vertebrates, and that they employed certain hormonal mechanisms in their reproductive economy; and he attempted to bridge the gap between sex-determining mechanisms dependent upon gene action alone in invertebrates and the current conception of hormonal influences

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in sexual differentiation in the vertebrates; it rather left the vertebrate sexologists desirous of more pabulum. Since investigations on hormones have been so intensively pursued for the past decade, impelled onward by the magnificent performance of biologists and biochemists in isolating, testing, purifying, analyzing and finally synthesizing many of the sex hormones, and since especially the writer has recently published observations and conclusions that to many are either unorthodox or downright heresy, this presentation may be regarded as the promised further discussion of the general aspects of the problem pertaining to the role played by sex hormones in sex differentiation (Moore, 1943).

Relatively recently a number of excellent reviews or synthesizing articles have dealt with the question of the role of sex hormones in sexual differentiation; these have applied to special groups or classes of vertebrates or have dealt with such special topics in the field as sex reversals, modifications of sex characters and others; but in view of their comprehensive documentation it is unnecessary to attempt complete coverage of relevant literature (Danforth, 1939, 1942; Domm, 1937, 1939; Willier, 1939, 1942; Witschi, 1939, 1940, 1942; Humphrey, 1942; Greene, 1942; Burns, 1938a, 1942a, b). A consideration of these reviews throws upon the individual a seemingly impossible task of clarifying existing opinions as to the meaning or significance of a vast multitude of factual materials. Confusion exists not only from lack of similarity in objective results or real differences in interpretation, but also mere terminology itself introduces considerable difficulty. Thus in articles presented in one symposium we may contrast the two following general statements:

> The most important general result evolving from recent work on sex determination in amphibians is probably a realization that under normal conditions the *sex hormones*[2] have relatively little, if anything, to do with primary sex differentiation, and that the inductor substances, which play their roles at earlier stages of the ontogenetic process than the sex hormones, belong in a separate class of chemical materials (Witschi, 1942).

[2] Italics, mine.

From another:

> The actual mechanisms whereby one sex component predominates over the other in the differentiation of the gonad appears to be endocrine in nature. This is clearly indicated by the sex reversal effects produced by the *sex hormones*[2] injected. What the genes actually control physiologically, therefore, is the intensity of hormone secretion. The gonad component with the relatively greater intensity of hormone secretion determines the direction of sex differentiation (Willier, 1942).

Despite these seeming contradictions, however, it appears that the authors may actually hold quite similar opinions relative to the subject. Differences in usage of the term "sex hormones" have been the cause of much misunderstanding.

Since the first demonstration of an internal secretion for the testicle by Berthold in 1849 we have spoken and written of the testis hormone, or male sex hormone. Steinach early in the present century firmly implanted into the literature the terms male, or testis hormone, and the female, or ovarian hormone, and endowed each of these with the two cardinal functions of stimulation of homologous tissues, and inhibition of tissues or end organs of the opposite, or heterologous sex; inhibition of the opposite sexed gonad was also believed to occur. As advances were made in the isolation of the respective active substances from the gonads it became apparent that several substances were obtainable from female tissues or fluids that exerted typical feminizing effects and from male tissues or fluids different pure chemical substances were prepared that exercised masculinizing reactions. These substances have come to be designated as estrogens and androgens. When the pure crystalline substance testosterone was prepared from testis tissue extracts and the parent substance was combined with other substances to produce the score or more chemical androgens, interests naturally centered on their biological activities. In the numerous instances wherein responses to androgens were given by a female end organ the tendency arose to speak of the estrogenicity of androgens, and similarly the androgenicity of estrogens.

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With all the tremendous advances in our knowledge we still do not know the constitution of the substance, or substances, secreted by the sex glands as their internal secretion, but presume the testis hormone to be somewhat similar to testosterone, or some compound of it, and the ovarian hormone to be similar to estradiol. We do not know whether the same hormonal substance is secreted by the different species, and there is still great uncertainty as to the period in ontogeny when hormone secretion begins, or when it becomes effective in the organism. Finally, we are not certain whether the gonad secretes more than one estrogen or androgen, but in some instances there is good evidence that the gonad may secrete both androgenic and estrogenic substances.

Recognizing all the attendant uncertainties and difficulties involved, however, it is of interest to consider the question of the role of sex hormones in sex differentiation, and particularly to examine somewhat critically the current viewpoints on the subject and survey the evidence for them. For the sake of clarity it may be stated that this discussion will be confined largely to the steroidal group of hormones that exercise such functions as are usually described under the terms androgenic and estrogenic.

The present generally accepted interpretation of sexual differentiation involves the notion of a genic mechanism in which, by virtue of the character of the parent germ cells, a dominance of the male-producing or female-producing set of factors is established. Although factors for both maleness and femaleness are present the more powerful set predominates over the lesser and gives character to the gonad as it develops into a testis or an ovary. An integral part of the sex equipments is the two sets of embryonic ducts—the male or Wolffian, and the female or Mullerian, duct—and other accessory organs. When the dominant set of sex factors expresses itself in the gonad as testis or ovary, specific hormones are believed to be secreted by the gonad, and the develop-

ment of the duct system and accessory sex characters is believed to be dependent upon the action of the hormone secreted by that gonad; the hormone would thus stimulate the homologous characters and inhibit the heterologous ones. Although enjoying wide acceptance there is very little substantial evidence to support this hormone interpretation aside from the case of the freemartin, which we may briefly consider.

In 1917 through the observations of Lillie (1917) it became known that twins in cattle experience fusion of embryonic membranes, together with fusion of allantoic blood vessels from the two embryos. This union of embryonic blood vessels, which permits an interchange of fetal blood between the members of the pair, is accompanied by a modification in the reproductive systems of females twinned with males, but not in any other twin combinations; no modification occurs in the reproductive system of the male partner. Presumably, a testis hormone secreted by the male is carried to the female partner and exercises its postulated double effect, *i.e.,* the male hormone inhibits the heterologous or female components of the developing female embryo, and stimulates the homologous or potential male structures. Thus the genetic female suffers inhibition, even recession, of the cortical portion of the ovary, and the medulla in many cases develops into a typical sterile testis; the Mullerian or female duct system is inhibited, and largely removed, and the Wolffian or male duct system is stimulated to develop, so that the unusual female (freemartin) becomes provided with an almost complete male reproductive system.

The remarkably clear and consistent analysis of this centuries-old freemartin problem, and Lillie's preferred interpretation of events on a hormonal basis (other possibilities were considered by him) at a period considerably earlier than the isolation of any of the sex hormones, represents both a masterful analytical investigation and the beginning of the conception that sex hormones play

an important role during sexual differentiation. This work not only represents one of the milestones along the pathway to a better understanding of some of the basic phases of the biology of sex, but also it has served as a powerful stimulant and challenge to investigators in this field for more than a quarter-century, and the theoretical aspects still represent the preferred interpretation of events during sexual differentiation. We find in the conception the basis of an explanation of human hermaphroditic conditions (Young, 1937) as well as sexual abnormalities of many distinct grades occurring among the different classes of vertebrates (Ivy, Greene and Burrill, 1939; Burrill, Greene and Ivy, 1940). The conception also remains basic for interpretation of modifications obtained after experimental treatments of embryos with the more recently available chemical hormones.

Recent observations of the writer, after experimental treatment of embryos, presented difficulties in interpretation on the basis of the hormonal control of sexual differentiation, and the failure to duplicate the essential freemartin effects in the course of more than twenty-five years of repeated attempts appeared to warrant a critical reexamination of facts and theories that have accumulated during the intervening years. This appears to be especially needed for interpretation of results reported after treatments with chemical hormones. In our ardor to learn all the possible effects of a hormonal substance there is danger of fixedly attending to the powers of the given hormone and of neglecting to consider broadly the powers of the organism to show changes under unusual conditions, or after unusual treatments. All effects of introduced hormones are based upon organ responses, and we sometimes neglect to consider that a response depends as much upon capacities of organs or tissues to respond as it does upon the agent administered. Intensive attention to one substance, or types of substances, may cause us to conclude erroneously that the reaction is a specific one whereas it may possibly represent a

response that may be induced by many totally different kinds of agents. It appears advisable, therefore, to momentarily back away from the immediacy of an experiment and attempt to inquire more broadly into the concept of the role of sex hormones in development.

Before entering into some detail on separate phases of investigation that have a bearing on the general problem, brief mention of somewhat similar conditions to that inherent in the fusion of membranes in cattle twins may be in order.

The character of the embryonic membranes in cattle appears to favor fusions between those present in the same uterus, and it might be presumed that other ungulates would show similar trends. The thousands of pregnant uteri of the pig examined each year in courses in embryology, where simple placentae may be under study, suggests that the usual necrotic ends of chorionic vesicles effectively prohibit fusion, and it is largely the exceptional side-to-side fusions which provide possible materials for study. Such were the types utilized by Miss Hughes (1927, 1929) in her study of a few cases of freemartin conditions in swine, as well as the single case of Hoadley (1928). Hughes points out a greater variability in the female modifications in fused pairs of heterosexual pigs than in cattle, and suggests that rather than complete cortical degeneration in the ovary the germinal epithelium retains considerable activity. Concerning such modifications as attend on blood vessel fusion between heterosexual twins Wislocki has reported two different cases in which expected results were not apparent. In twin cat fetuses fusion between fetal blood vessels of a heterosexual pair had no apparent effect upon the reproductive system of either sex (Wislocki and Hamlett, 1934); in the marmoset a few heterosexual pairs showing well-developed blood vessel connections did not show any trace of the modifications that are of usual occurrence in the female of cattle although the structural union was such as to expect them (Wislocki, 1939).

Such departures from the expected results suggest that among other things we should be careful in making assumptions that one species reaction will be the same in other species. We are yet unable to state whether cattle present some specific or special condition that favors the responses in the female of a heterosexual pair, but aside from the cow the freemartin type of influence is known to occur only occasionally in the pig and possibly goat.

Three general lines of approach have been employed to put to an experimental test the conceptions inherent in the theory of hormonal control of sex differentiation. These may be mentioned as evidence from (a) utilization of living tissues, (b) utilization of chemical hormones and (c) evidence provided by gonadectomy. These we may briefly survey in turn.

Evidence from Utilization of Living Tissues

In mammals, attempts have been made many times to simulate the basic freemartin conditions through the transplantation of developing gonads into opposite sexed hosts with the hope that successful testis transplants might introduce testis hormone into differentiating females. The reverse, or transplantation of developing female reproductive tissues into differentiating male hosts, sought to provide similar conditions in which developing female tissues would be subjected to male hormone secretions. A further procedure, employed to effect possible exposure of developing females to male sex hormones, was the method of parabiosis; this has been largely unproductive for the particular problem where mammals have been used, but in the case of amphibia it has been exceptionally productive in some phases which, as will be pointed out later, are largely inapplicable to the sex hormone conception of modification.

Approximately fifteen years ago the writer, using mammals, made numerous attempts to transplant testes as grafts into embryos of known age in rats and guinea pigs by opening the uterus and embryonic membranes.

These efforts, as well as attempts to introduce testis hormone into embryos by breeding females carrying active testis grafts, were unproductive with respect to our present problem of interest although they yielded important information for other problems. Parabiotic union between adult male and female, with attempts to breed the female of the pair, likewise failed to yield pertinent information; other workers have obtained offspring from females in parabiotic union with males without reporting sexual modification in offspring.

Transplantation of female embryonic reproductive tissues into intact male hosts of early ages provided more hopeful conditions for realizing modifications through circulating hormones. Buyse (1935) transplanted posterior portions of rat embryos of 11 to 14 days of gestation into the kidneys of adult males and females, and Moore and Price (1942) transplanted gonads, gonads and ducts, or the duct system alone, dissected from rat embryos from the 15th to 19th day post-coitum into subcutaneous pockets in post-natal hosts of age 4 to 30 days. On the study of gonadal development these two investigations are in agreement that as extremes, ovarian differentiation occurring in male hosts may result in normal appearing cortical tissue containing follicles, or in the conversion of medullary cords into testis-like tubules, but neither investigation provided evidence for a hormonal modification of gonad development; grafts resident in male or female hosts showed similar ranges in development. Duct differentiation, considered by Moore and Price, also failed to provide any response suggestive of a freemartin type of development, since neither obvious stimulation nor depression from heterologous hormones was in evidence.

In the bird the experiments of Willier (1927) would presume to provide adequate relations for embryonic hormone effectiveness on differentiating gonads and ducts. Chick gonads, either in the morphologically indifferent stage of development or the sexually differen-

tiated stage (4 to 13th day incubation), were transplanted to the chorio-allantoic membranes of hosts incubated for 9 days. The successful grafts, recovered on the 18th day of host incubation, were therefore vascularized by host blood vessels, but, irrespective of the sex combination of host and graft, modifications in sexual differentiation were not noted; transplanted ovaries, vascularized by male host blood stream, differentiated normally, and female hosts carrying well-incorporated testis grafts in the membranes were not modified. These observations led Willier to conclude that "Hormonic sex-differentiating factors of the host embryo are either absent or, if present, they are ineffective in the modification of the engrafted sexual glands." The experiments of Dennis (1936) imposed even more rigorous conditions on both host and differentiating graft since not only were ovaries, prior to morphological differentiation, transplanted on the chorio-allantois of male hosts but they were accompanied by a testis graft on the membrane, and yet failed to indicate any male hormone influence; such associated testis grafts might be in actual contact with the differentiating ovary graft, and the testis graft might have originated from testis tissue known to be secreting hormone at the time of transplantation. Multiple testis grafts on the membranes failed to modify a female host supporting them.

Thus in birds and mammals, aside from differentiating embryos of pigs, cattle and possibly the goat, only negative evidence of modification of reproductive tissues (gonad or duct system) during the period of sex differentiation has emerged. Since response of tissues is the only criterion to indicate the presence of hormone it appears justifiable to suggest, also, that no evidence has been forthcoming to indicate hormone secretion by a differentiating gonad among these experimental procedures in which living tissues have been depended upon to supply hormone.

Amphibia have been the subjects of numerous experimental procedures devised to study the effects on sexual

differentiation of hormones derived from living tissues. Such studies, carried on principally in this country by Witschi, Burns and Humphrey, have involved the transplantation of undifferentiated or sexually differentiated gonads into larvae of the opposite genetically determined sex, of the same or different species or race, as well as extensive experiments on parabiotic union of the same or opposite sexes. Recent very complete reviews of these studies (Witschi, 1939, 1940; Humphrey, 1942; Willier, 1939; Burns, 1938a) are recommended to the interested reader and the recall of outstanding results may serve for the present discussion. When the larval testis of a urodele, in the pre-primodial or primordial stage, is transplanted into a genetically determined female the developing host ovary undergoes such changes in the cortex-medullary components as to become a structural testis-like organ; a similar result may be obtained when heterosexual larvae are united in parabiosis. The cortex of the ovary suffers inhibition and the medulla is able to differentiate as a testis; the effective substance in the urodele appears to be carried by the circulatory system. Conditions in frogs and toads, while leading to a similar general interpretation that hormone-like substances are specifically produced in the two components of the sex gland ("cortexin" and "medullarin" provided by the cortex and medulla, respectively) that are mutually antagonistic, differ with respect to the intensity and distance over which the alleged substances are effective; in some species of frogs the substances are believed to diffuse through the surrounding tissues rather than being transported by the blood stream, and in the toad the range of effectiveness appears limited to the gonad itself. The medullary influence largely predominates over the cortex and effects transformation of ovaries to testes, but in certain combinations the cortical influence may predominate and lead to the formation of an ovary-like organ in the genetically determined male.

Our primary interest here is the bearing these phenomena have on the problem of the role of sex hormones

on sex differentiation. For some years Witschi has been insistent in separating such effects as those reported above for larval amphibia from the true sex hormonal mechanism which, in amphibia, is evident at later periods in development; in most of these so-called "inductor" phenomena the duct system has not been involved. It is thought that these earlier modifications of gonads by living tissue may depend upon hormonal-like substances, presumably of a protein nature, entirely separate from the steroidal class of hormones with which we are especially concerned. Further consideration of this phase will be given below. On the whole, amphibia have demonstrated a very labile type of sex factor balance, and shifts from one condition of the gonad to another in addition to being effected by such experiments with living tissues, are likewise encountered in overripeness of eggs prior to fertilization, in treatments with lower or higher than normal temperatures, and probably in many other types of treatment; they, therefore, can not be regarded as specific influences but only as results that may follow many types of treatment. Hermaphroditism among amphibia is of relatively frequent occurrence and gametes of both types can function in fertilization although the genetical character of the original zygote is retained despite the reversal of gonad to the opposite sex type.

Evidence from Chemical Hormones

The availability of pure chemical androgens and estrogens, as well as effective preparations of gonadotropic substances, has led to the wide use of such substances in studies on sexual development in the five classes of vertebrates. These experiments have yielded most interesting results on the capabilities of the tissues to respond to hormones, and to show modifications in sexual development, but the value of the information for an understanding, or analysis of normal processes controlling sex differentiation has been variously assessed. Numerous modifications in sex differentiation have been reported

following treatments with the chemical hormones, but many failures to reveal modification under apparently similar conditions in other species are likewise recorded. Frequently the responses are non-specific, and may be recorded as paradoxical, and it is just possible that their greatest significance may lie in the emphasis on the capabilities of the organism to develop in devious ways under handicap; insults to developing tissues may demonstrate the well-recognized capacity to differentiate sexually in one of the two possible alternative manners, or to show a mixture of the two types.

Fishes: The young females of *Xiphophorus helleri* subjected to treatment with androgens shortly after birth are reported to show resorption of large eggs in the ovary, with ovaries gradually assuming testis-like appearance, but spermatogenesis was not noted (Witschi and Crown, 1937); others report 50 per cent. of the females developing into a male type organism with spermatogenesis indicated (Baldwin and Goldin, 1939). The minnow, *Phoxinus laevis,* exhibited a breakdown of the ovary with the assumption of a testis-like gonad (Bullough, 1940), whereas ovaries of the minnow, *Lebistes reticulatus,* showed suppression of growth and ovogenesis (Eversole, 1939, 1941).

In treatments with estrogens the male guppy, *Lebistes reticulatus,* experienced suppression of the male gonopodium and spermatogenesis was modified or suppressed. A mosaic of male-female characters was induced, as well as an ovo-testis; almost complete reversal of testis to ovary occurred (Berkowitz, 1937, 1941).

Responses of these types suggest a rather specific modification of the sexual state appropriate to the type of hormonal substance employed, or the induction of maleness by androgens and femaleness by estrogens. However, treatments of *Lebistes* with the synthetic steroid pregneninolone, which exhibits progestational activity and little or no androgenic activity, lead to an inhibition of female characteristics and a stimulation of the male

gonopodium and in males a precocious maturation of the male elements (Eversole, 1939, 1941). Such responses suggest strong androgenic effects, but since this substance as usually tested on higher forms largely lacks such an action, it becomes of interest to speculate whether the responses in the organism are to those portions of the compound responsible for its androgenicity or merely to some type of chemical agent that disturbs the normal physiological processes in the organism. Witschi (1942) calls attention to unpublished observations of his student Crown (1941) that estrogenic compounds masculinized *Xiphophorus* females—a response just the opposite to expectation; ovaries regressed, and fins transformed into a gonopodium and the male sword began formation and growth. Baldwin and Li (1942) point to somewhat similar responses on the part of these females when treated with extracts from pregnancy urine; virgin females under such estrogenic treatment developed a male gonopodium, exhibited a male copulatory pattern and a reversed sex coloration and various stages of spermatogenesis were present. Without the use of any treatment, other than surgical removal of ovaries, the female fighting fish (*Betta splendens*) regenerated gonads of testis-like character, produced spermatozoa, and developed fins of the male type (Noble and Kumpf, 1937); and parturient *Xiphophorus* females are believed to frequently undergo sex reversal to the male condition, and produce spermatozoa, while living under natural conditions and without treatment (Essenberg, 1926).

This brief review of the unstable and changeable capacity of fishes to show different sexual states under treatment with hormones, or under other circumstances, leads one to question the significance of the androgenicity or estrogenicity of a substance to effect specific sexual responses in fish. Whereas some changes induced are possibly the expected ones in terms of the hormone substance employed, others distinctly are not; in addition, even more remarkable changes may occur without any

treatment with hormonal substances. Perhaps the results are of value merely to emphasize the unstable sexual condition in many fish, and that disturbances of normal physiological processes under which the genic tendencies for sex express themselves, may be easily induced by hormones and perhaps by many other non-hormonal substances. When non-hormonal agents are employed in experiment with as great industry and interest as have the chemical hormones we may find expressions of androgenicity and estrogenicity from very unexpected substances or conditions.

Amphibians: Turning to the amphibia for an examination of effects of chemical hormones on sex differentiation we meet another conflicting set of results. In the main, when sexual modifications have been induced through such treatments, it is rather frequent that treatments of females with androgenic substances tend to modify these in the direction of maleness, and males treated with estrogens go over towards sexual differentiation of females. Thus androgenic treatment of females leads to the differentiation of a testis or testis-like gonad in *Amblystoma punctatum* (Burns, 1938), *Rana clamitans* (Foote and Witschi, 1939), *R. pipiens* (Foote, 1938) and in *R. catesbiana* when treated simultaneously with a gonadotropic agent (Puckett, 1939, 1940). Estrogenic treatments lead to feminizing influences on males and may convert the genetically destined testis to develop as an ovary, or ovary-like body, in *Amblystoma* (Ackart and Leavy, 1939; Burns, 1938b; Foote, 1940 and 1941), and in *R. catesbiana* if treated simultaneously with gonadotropins (Puckett, 1939, 1940); but when we recall similar modifications induced by living testicular and ovarian tissues, reported in the previous section, and the belief that the active agents in such transformations were substances of protein-like nature, one is in a quandary in attempting to evaluate the significance of the responses to the steroidal sex hormones.

In addition to these reversal effects that tend to conform to the nature of the hormonal substance—*i.e.*, androgens and maleness, estrogens and femaleness—other responses have been seen that do not follow such a pattern of correlation. *Rana pipiens* larvae treated during the indifferent stage with the synthetic steroidal compound exhibiting progestational effects (pregneninolone) are found later to be 100 per cent. males (Eversole and D'Angelo, 1943). Androgens may exert femininizing effects instead of the expected masculinizing ones (Ackart and Leavy, 1939; Burns, 1938b; Foote, 1940, 1941) and estrogens may induce masculinizing modifications rather than femininizing (Foote, 1938); and in several different experiments androgen or estrogen treatment may not lead to any modifications of the developing gonads. These diverse effects from chemical hormone treatment, coupled with known tendencies of overripeness of eggs to induce maleness, the tendency for lower than normal temperatures to favor femaleness and higher than normal temperatures to favor development of male tendencies in the gonads naturally introduces a quandary relative to the significance of these findings as indicating that specific actions of hormones are important in the differentiation of sex. Rather to be emphasized, it would seem, is the great liability toward sexual differentiation exhibited by the amphibia. The different conditions tend to suggest a lack of specificity of such agents and the probability that many other types of treatment that would interfere with ordinary physiological processes under which the expression of genic determination is to be seen might be equally effective.

Reptiles: Although reptilian materials have been less frequently examined for sexual modifications after treatment with sex hormones several reports are pertinent to the present discussion. Through the injection of chemical hormones into developing eggs of the lizard and turtle, and into young alligators shortly after hatching, such modifications during the course of embryonic devel-

opment have been seen as to cause some investigators to explain sex differentiation on the basis of internal secretions occurring in the embryo. In the lizard, Dantschakoff (1938) demonstrated that estrogenic treatment during development induced modifications and that in both sexes cortical development in the gonad predominated at the expense of the medulla; on account of these feminizing tendencies, especially in males, she considered that the chromosomes produce a specific type of hormone which directs development of the indifferent gonad into the proper organ, testis or ovary. Risley (1940, 1941) noted also in the lizard that following androgenic treatment the medulla in the ovary was enlarged and became testis-like; this is regarded, however, as merely a secondary response of this tissue after differentiation of the gonad has already occurred under ordinary genic influences, hence hormones do not replace the induction mechanism inherent in the genic constitution. The accessory reproductive organs failed to exhibit any response. When he employed the recently hatched diamond-back terrapin Risley did not find a tendency for changes to follow in correspondence with the androgen or estrogen employed; gonadal changes were minor and both Wolffian and Mullerian ducts responded to treatment with androgens and estrogens. These results led him to subscribe to the notion that such hormones were not the agents controlling normal sex differentiation. Young alligators showed slight tendencies for stimulation of the cortex in both testes and ovaries by estrone (Forbes, 1937, 1939). The female ducts responded readily to both androgens and estrogens hence specificity of hormone action, inherent in the hormone concept of sex differentiation, appears not to hold for reptiles.

Birds: The treatment of the developing chick with chemical hormones (Wolfe and Ginglinger, 1935; Dantschakoff, 1936; Breneman, 1935; Willier, Rawles, Gallagher and Koch, 1937, 1938) has resulted in many types of modification in the reproductive system and

some writers have seen in these responses evidence that the control of sex differentiation under normal conditions is probably exercised by hormones secreted by the developing gonad; a few of these conflicting results may be recalled.

In the chick, estrogens injected into eggs incubated for 48 hours, with embryos preserved on the 18th day of incubation, exert but little effect upon developing females; the ovary is not perceptibly modified and only with higher dosages the oviducts may exhibit hypertrophy with some increase in length of the right reduced oviduct. In developing males, however, marked changes appear following estrogenic treatments. Testicular modification differs materially in the right and left gonad since the right testis is usually unmodified excepting with the highest doses. The left testis exhibits all grades of differentiation from a normal testis to a gonad that is essentially ovarian in structure; the cortex reveals varying grades of stimulation and the medulla all grades of inhibition, or modification of the usual solid testicular cords into characteristically open and distended ovarian medullary cords. The oviducts of such males may persist partially or completely, the right duct being either slightly enlarged or greatly hypertrophied.

When androgens are utilized in the treatment the developing chick may exhibit a number of, and somewhat curious, responses depending upon the nature of the androgen employed (androsterone or testosterone), the genetic sex of the embryo and the dosage employed. Female chicks may respond to androgen treatment by a gradual degeneration of cortex and hypertrophy of medulla leading to an organ largely testicular in appearance; even the normally reduced right ovary may reveal hypertrophy of the medulla, hence the female chick is highly modfied in the male direction. The left oviduct may be reduced to a short rudimentary ostial portion while at the same time the right oviduct, normally reduced or absent, may be preserved and show a great

increase in length. Male chicks treated with androgens revealed gonads that were only slightly reduced in size when the androgen was T-propionate, but when the androgen employed was androsterone the testis was almost completely changed over to an ovary. The left testis responded by cortical hypertrophy and testis cords were changed to resemble ovarian medulla. Thus it follows that the cortex of the genetic ovary is *inhibited* whereas its homologue in the genetic testis is *stimulated.*

The oviducts in males treated with the androgen T-propionate are not preserved but those in males treated with androsterone not only persist but may also exhibit a great increase in size. Further inconsistencies appear in that the oviducts, in genetic females treated with androgens, may show in the same embryo a reduction in the left oviduct and a stimulation in the right one.

Thus, these readily produced responses in developing embryos treated with chemical hormones are of very great interest in revealing the capacities of genetically determined sexes to exhibit characteristics belonging to the opposite genotype, but to apply the experimental results to normal development as an indication that sex differentiation is controlled by androgens or estrogens secreted by the genetically determined gonad would appear to be an impossible task. The differences seen in the two sexes do emphasize the power of the inherent genetic constitution to regulate the capacities for response on the part of the tissues to the substances introduced. But in the estimation of the reviewer they cannot be taken as evidence either that sex hormones are involved in the differentiation of the sexual equipment, nor indeed as evidence that the gonads are actually secreting. It will be recalled that Willier and Yuh (1928) have shown radical modifications in sexual differentiation in chicks that have carried muscle, or thyroid, grafts on chorio-allantoic membranes; and that even mere chilling of eggs increases atypical sexual modifications. These authors accounted for modifications described by Minoura

(1921), and attributed by him to sex hormones, as nonspecific effects probably unassociated with gonadal hormones. Willier (1927) has emphasized the lack of influence on differentiating gonads exposed to the constant circulation of an opposite sexed host, hence to any circulating hormones that would be present in the event the intact host gonads were secreting.

It has been shown that chicks treated with gonadotropic substances during embryonic stages experience an increase in size of gonads but fail to give evidence for hormone secretion (Domm and Dennis, 1937); following treatments begun at very early periods, and also at later periods, no hypertrophy of Wolffian or Mullerian ducts appeared. Had the gonads been capable of secreting hormones at such periods, and especially since direct application of androgens and estrogens have proved these structures capable of response, duct reactions should have indicated it, hence it is suggested that the gonads of developing chick embryos are not secreting hormones; gonadotropic treatment of chicks immediately after hatching does clearly reveal the capacities for gonadal hormone secretion hence this capacity to respond must be attained sometime around the period of hatching. The reproductive systems are already well developed, however, hence sex hormone secretion by the gonads could not well be presumed to have been operative around the 10th day of incubation and earlier, as would be required, were they made responsible for controlling differentiation of sex ducts.

Mammals: The developing mammal has also been subjected to treatments with chemical hormones and sexual modifications have been described in the treated mouse, rat, guinea pig and opossum (Dantschakoff, 1937; Burns, 1939; Greene, Burrill and Ivy, 1939, 1940, 1942; Hamilton and Wolfe, 1938; Raynaud, 1938; Turner, 1939; Moore, 1941). Great variability in the number and character of responses has been pointed out. Strikingly absent have been modifications in the character of the gonads during

their differentiation; it would almost seem as though the genetic mechanism for sex differentiation was markedly stronger than exists in the lower vertebrates, and that the gonads have gradually lost their great lability, or power of potentially different developmental capacities, as evolution has advanced. Neither the cortex nor medulla in any of the species cited has shown a definite change from normal development when subjected to estrogens and androgens. Testes in some cases have suffered some interference with their normal growth rate and have failed to descend into the scrotum, frequently in association with enlargement of duct portions believed to interfere with descent; ovaries in some cases appear to reside in somewhat more posterior positions as if there was the testicular tendency to backward descent. In no case, however, has an ovary, developing under the influence of androgens, shown any tendency to respond as did the freemartin ovary, by cortical degeneration and medullary development.

Duct and accessory reproductive organs have shown decided modifications after treatments with chemical hormones but in the aggregate the responses have been too heterogeneous and non-specific to suggest any credence in the belief that the developing gonad secretes similar substances that account for normal development. Thus the responses noted earlier in the investigations suggested evidence that would fit the postulates for specific action of hormones on duct development, since treatments of pregnant rats and mice with androgens was followed by the retention in newborn females of male or Wolffian ducts. The second postulated function for the hormone was not evident, however, since the female reproductive system, aside from the distal third of the vagina, was normal whereas it should have been inhibited by the androgens; oviducts and uterine horns were entirely normal. It is clear that freemartin effects, as they apply to duct systems, were evident only in part. A greater unconformity was encountered when pregnant females were

treated with estrogens and developing females showed the unusual retention of Wolffian or male ducts, to an extent similar to that which followed treatments with androgens. In developing males, also, some features suggested conformity with the hypothesis of hormone control of duct development, whereas others failed to do so.

In a high percentage of treated pregnant mothers placental damage was marked (high fetal death and resorption just prior to parturition). It is conceivable, even probable, that such placental injury may of itself engender abnormal development and that atypical conditions might possibly be entirely separable from specific actions of the hormones employed. Such uncertainties were avoided by the utilization of the developing marsupial as the experimental subject. The opossum at birth is structurally undifferentiated as to sex, with gonads indistinguishable as ovary or testis, with large Wolffian (male) ducts functioning as urinary ducts, and with Mullerian or female ducts showing first appearance on day three, and requiring until about day ten to attain their urogenital sinus connections. During these periods of development, and later, opossum pouch young were treated daily with androgens and estrogens. Androgenic treatment caused but little stimulation of male (Wolffian) ducts, and would not preserve their posterior connections in females; persistence of portions of the Wolffian ducts, however, was more prevalent than in normal female development. In developing females these androgenic treatments not only failed to inhibit and eliminate the female ducts, as would be anticipated, but they caused marked precocious stimulation and induced uterine gland formation. It was noted that both the male and female duct systems, in either the genetic male or female, could respond to androgens or estrogens. It has been noted by Burns, however, that sufficiently weak doses of hormones can be utilized as will produce stimulation of the homologous duct system without stimulation occurring in the heterologous ducts; heterologous inhibitions were not

noted. It will be of much interest to see how far this type of reaction may be carried, and to determine whether it has greater significance than a different threshold of response in the two duct systems.

One feature was encountered in these experimental procedures that is believed to be of special importance. It was found that when litter-mate males and females were treated with androgens during the early stages in formation of the Mullerian (female) ducts, the responsiveness of this duct was markedly different in the two sexes; daily treatments with the same dose of androgens, up to approximately day 30, revealed that the diameter of the lumen of Mullerian ducts in females was 100 times that in males. This fact emphasizes that the genetic constitution of the organism, irrespective of our notions of morphological homologies, clearly determines its capabilities of response to inciting agents; certainly the capacity of the Mullerian ducts to respond to the same agent is totally different in the two generic sexes, and it focuses attention upon the importance of genetical sex determination. Burns has repeatedly pointed to instances in which differential responses of the same structure occur in the two different sexes.

Since the capabilities of developing structures to respond to androgens and estrogens were so apparent, young male and female opossums were subjected to treatments with gonadotropic substances in an attempt to determine whether developing gonads were secreting sex hormones, or were capable of doing so (Moore and Morgan, 1943). When the duct system in females, and prostate gland development in males, were employed as indicators for estrogens or androgens liberated by a stimulated gonad it was found that evidence for testicular hormone secretion was obtained at an age of approximately 60 days or older, whereas no evidence was had for ovarian hormone secretion until subsequent to day 100; some evidence has more recently been obtained for an earlier testicular response when Cowper's gland is uti-

lized as indicator for androgenic substances (Rubin, 1943), but all the evidence thus far available suggests that the developing gonads are incapable of secreting sex hormones when subjected to treatments that should cause them to secrete, if capable, until periods much later in development than sex duct differentiation.

The problem of the stage in development at which different portions of the reproductive system are capable of responding to chemical hormone influence is recognized. The problem has recently been investigated by Price and Ortiz (in press) in young rats. With standard dosage and constant length of treatment, and age at beginning of treatment as the variable, it has been found that male tissues in general reveal a low responsiveness to androgenic treatment at birth, and that sensitivity increased rapidly to a peak around day 30 when normal males usually reveal changes leading up to puberty; similar conditions for responses of female tissues to estrogens was also noted, but the initial response to treatment was relatively greater in females than in males. Hormonal secretion by the gonads, as a response to gonadotropic treatments, also exhibited a gradual increase from birth up to three or four weeks of age. In the investigations of Smith and Engle (1927) it was pointed out that pituitary stimulation of ovaries was readily evoked around day 20 but much less evident prior to this period. These, and many other, collective evidences suggest that the province of hormonal control over reproductive tissues, and functions, is properly one that emerges with gradual development around the natural age of puberty, and other indications equally suggest that hormonal actions are probably not normal controlling agencies during early establishment of the tissues that will later be the responding end organs when the hormonal mechanism becomes established.

EVIDENCE FROM GONADECTOMY

The final body of evidence to be discussed for its bearing on the relation of sex differentiation to hormones, and

the question of the onset of hormone secretion by gonads, will be drawn from normal prepuberal development, and especially from the effects of early gonadectomy.

The general features attendant on the attainment of puberty are fairly well known. The mention of a few specific events, however, may aid in more sharply focussing attention on the earlier evidences for sex hormone secretion. Such events, that depend upon gonad hormone secretion, as growth of breast tissues and the first menstruation afford definite signs of approach to puberty in the human female some years after birth. The first vaginal introitus occurring in rats and mice, at ages variable in colonies and strains from day 35 to 65, indicate the first outpouring of hormones in sufficient concentration to effect this cyclic event. Young male rats about 35 days of age reveal the early signs of secretion in seminal vesicles, which is definite evidence for the attainment of hormonal secretions sufficient to accomplish this stage in development. Guinea pigs first produce coagulable ejeculates (indicating prostate and seminal vesicle function) around day 20 (Sayles, 1939). Such data provide us with the earlier indications in normal development for detectable beginning of sex hormone secretion, and numerous experimental treatments with gonadotropins clearly demonstrate capabilities of gonads to secrete earlier, or more intensively, than occurs in normal development.

Gonadectomies performed at very early periods should by the removal of all possible gonad hormone secretions provide further evidence relative to hormone secretion. Wiesner (1935) castrated male and female rats at birth and considered that he was able to demonstrate hormonal influence from about the 10th day after birth in the male, but not until the approach of puberty in the female. On the basis of these and other procedures he was one of the first to question the correctness of the conception of the hormonal control of sex differentiation, and developed the hypothesis that females differentiate without the

influence of sex hormones whereas the male requires testis hormone to effect its differentiation. This "monhormonic" theory visualized all reproductive differentiation to take place in the female direction if hormones were not present, and that under the influence of testis hormone, development was modified into the male pattern. Price (1936) castrated male rats at birth and, on the basis of prostate gland and seminal vesicle histology, was able to note deficiencies in male hormone as early as the 11th day; and in unpublished observations of Cieslak, who employed weights of epididymis in young rats as the indicator, deficiencies are observable by the eighth or ninth day after birth. Collectively, these findings suggest that testis hormone secretion in the rat is apparent very shortly after birth. In any event it is to be expected that hormone secretion is a gradual development, perhaps beginning at a very low intensity and gradually becoming stronger, and it is not until sufficient concentrations are present to affect responding tissues that it becomes evident. We should, however, keep clearly in mind that the gonads are capable of hormone secretion prior to the time it can be detected normally, since treatments with gonadotropic agents can incite secretion considerably earlier than it is apparent in untreated animals.

The time in development at which gonadectomy can be accomplished in placental mammals is thus far limited to post-natal ages but in the marsupial, although also limited to a post-natal period, gonadectomy can be accomplished during stages in development that are present in placental mammals considerably earlier than birth; in effect, such a procedure is the equivalent of "embryonic castration." In the opossum, ovaries and testes have been removed on day 20 while the young are confined to the marsupial pouch firmly fixed to the nipples (Moore, 1943). The prostate gland in males and the reproductive tract in females, indicators for presence or absence of hormones, are still in the anlagen stage; prostate gland is represented by a few knob-like outgrowths from the

epithelium of the urogenital sinus, and Mullerian ducts in females are simple undifferentiated straight tubes. Following the removal of testes on day 20 prostate differentiation proceeded uninterruptedly in normal manner and rate until a period later than 100 days; similarly, ovariectomy on day 20 was followed by characteristic differentiation of the simple Mullerian ducts into oviduct, uterus, and lateral vaginal canal up to periods later than the 100 day stage. Regression of the opposite, or heterologous, sex duct proceeded as in unoperated males and females. Since this differentiation of both male and female accessory reproductive structures was, in character and rate of development, the equivalent of that in normal controls, the results suggest two major conclusions: (1) Differentiation of the reproductive system continues up to well defined stages of completeness in the absence of gonads hence such development does not depend upon hormone secretion by the developing gonads, and (2) hormone secretion by the normal untreated gonads can be definitely detected only subsequent to an age of 100 days.

In a comparison of normal development of the opossum and rat, considering such elements as first appearance of a lumen in the seminiferous tubule, canalization of prostate out growths, uterine gland development, first ovarian antrum formation, and still others, the rat of 10 days of age compares favorably in sexual differentiation with the opossum of approximately 110 to 125 days of age. The slower development in the opossum, therefore, permits more definite analysis of hormone effects and development occurring without hormone secretion.

General Summary

In this discussion an attempt has been made to bring to attention some of the considerations required for proper orientation to the problem of whether sex hormones play a role in sexual differentiation. As leading to a clarification of the central problem, and with attempts to avoid

so many of the current confusions, it appears advisable to restrict the general term "sex hormones" to that conception which is most appropriate from the historical usage. Sex hormones, therefore, should be limited to the secretions, of a steroidal nature, that are provided through activity of the gonads, designated as androgens and estrogens, and which play a distinct role in the control of morphology and physiology of the accessory organs of reproduction; their natural province of action is the reproductive system, aside from the gonads, and on such characters as plumage or superficial excrescences, and on behavior; some investigators will still question whether these hormones influence the organs which produce them—the gonads themselves. We would thus restrict our application of such terms as sex hormones, gonad hormones, testicular and ovarian hormones, cortical and medullary hormones, and it then becomes a problem to express our notions relative to their time of origin and sphere of operational influence. In turn, we would avoid the inclusion under the term sex hormones of substances produced by adrenals, pituitary, or other sources that might under some conditions exert an influence on sex characters, as well as such other substances possibly produced by chromosomes, or "inductor" substances presumed to express the action of male- and female-determining genes in producing cortex and medulla, and even substances that may possibly operate in a mutually antagonistic manner between cortex and medulla in the final establishment of the gonad. Witschi has designated by special name the hypothetical substances "cortexin" and "medullarin," has spoken of them as "inductor substances," has defined their sphere of action as intragonadal, and not affecting secondary sex characters; he regards these substances as possibly protein-like in nature. Whether the term "inductor" is appropriate for such substances is questioned by some on the basis that inductor actions usually imply a stimulating action whereas Witschi (1940) states

This interaction manifests itself mainly as a mutual antagonism; effects indicating positive stimulation have not been definitely demonstrated.

The term "inhibitor substances" used by Humphrey (1942) to explain the same phenomena appears less objectionable. Thus

The inhibitor substance in the Urodele is of the character of a hormone, since it is transported through the blood from testis to ovary in grafted animals, or from male to female in parabiotic twins.

Both Witschi and Humphrey exclude these substances from the sex hormones, and make clear that the sex hormones, produced at later periods in development, are responsible for the secondary sex characters during reproductive life. With this restriction, therefore, what may be said of the role of sex hormones in sex differentiation?

The application of chemical androgens and estrogens during development has induced many modifications, both with respect to the character of gonadal differentiation and in the differentiation of the sex ducts and other accessory sex characters. Some modification of gonadal differentiation after treatment with these chemical hormones has occurred in all groups, with the exception of the mammals, but the modifications have lacked specificity, conformity with the character of hormone, or predictable responses. Furthermore, the many other agents that may induce quite similar responses—in amphibia, temperature changes, overripeness, gonad grafts, non-androgenic or non-estrogenic synthetic steroids; in the chick, muscle, thyroid or other types of chorio-allantoic grafts, handling, chilling of eggs or other manipulations—suggest that where recognized androgens and estrogens have induced modifications the possibility exists that they exercise merely non-specific disturbances of the normal physiological, or genic, mechanisms; they may be no more specific, nor applicable to normal development than is shaking of eggs for the production of rumplessness in fowl (Landauer and Baumann, 1943).

On the duct systems, likewise, responses to androgenic and estrogenic treatments have been so variable and lack-

ing in specificity as to exclude the belief that substances of this nature could be responsible for controlling the normal course of sex differentiation. The responses have been of much general interest in demonstrating the capacity of the ducts to respond to hormones, and frequently to respond to either androgens or estrogens in a non-specific manner. The responses witnessed, therefore, do not lend encouragement to the idea that similar substances operate normally to regulate development of the reproductive system.

The utilization of gonadotropic agents, and early gonadectomies, serves to suggest that actual sex hormone secretion by the gonads begins considerably later in ontogeny than the characteristic disposition of the embryonic duct systems. The available evidence suggests that in the bird sex hormone secretion begins only after hatching, in the rat from birth up to the 10th day, in the guinea pig perhaps slightly later, in the opossum not until an age subsequent to the 100th day of post-natal life. If these suggestions can be admitted to possess any real merit, it would become necessary to exclude gonad hormones as agents in the regulation of sex differentiation of the reproductive system. Finally, the demonstration that gonad removal during the anlagen stage of the reproductive system in the opossum is without perceptible effect on differentiation of the sex ducts (Moore, 1943), and glands (Rubin, 1943), up to day 100, when differentiation is essentially complete, forces us to attend more seriously to the genic sex factor balance to effect differentiation of the reproductive system; the intervention of gonadal secreted hormones to guide these early normal processes is therefore not required.

As a closing consideration relative to the freemartin case, it can be said that a satisfactory interpretation is yet to be given. That the conditions found in this unusual type of development can be explained on the basis of sex hormones as the effective agents is not suggested by any demonstrated modification in mammals. That the testis

may be secreting hormones has not been disproved, but all evidence at present available brings it into grave doubt. That the effects of blood intermixture between heterosexual twin pairs are quite unique for ungulates, if not for the bovine species, is suggested. There exists a high degree of similarity in response of the gonadal, but not duct, system to that found in parabiotic urodeles. Responses of the duct system in the freemartin are not yet duplicated by any experimental treatment in mammalia, either under the influence of living tissues, or from treatments with the chemical androgens. It would appear incontrovertible that a humoral substance, transmitted through the blood stream, must be held accountable as the effective agent, but the nature of the substance is not indicated as being of the steroidal sex hormone type; furthermore, that it is a testis secreted substance appears to the writer doubtful, because of the lack of any supporting experimental results. Elsewhere (Moore, 1941) the suggestion has been offered that the chemical differences in the blood stream of genetically determined males and females, possibly from contributions of the cells of the soma tissues in general, rather than the testis specifically, may represent a conditioning agent. In any event, the freemartin still provides the challenge for proper interpretation that it presented more than a quarter-century ago.

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BREEDING *XENOPUS LAEVIS*[1]

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NUMEROUS inquiries have been directed to the Department of Animal Behavior, American Museum of Natural History, within the past year for information concerning the breeding of the South African clawed toad *Xenopus laevis*. The sudden interest in this toad is due mainly to its use as an indicator in the human pregnancy test. (For details of this test see papers by Elkan, 1938, Crew, 1939, and Weisman, Snyder and Coates, 1942a.) An investigation of the possibilities of breeding *Xenopus* in captivity was initiated in the Laboratory of Animal Behavior in 1940. Because of the slow rate at which the young mature, and because of the scarcity of new field material, the problem is far from complete. Nevertheless, because of the many requests, it was thought advisable to report at this time all available information. The following is a progress report of our observations and results, together with some practical advice to those about to undertake the breeding of this toad. A brief review of the literature is included.

The earliest account of the breeding of *Xenopus* which has come to our attention is that of Beddard (1894) who obtained viable eggs in the gardens of the London Zoological Society in 1894. A few years later Bles (1901) obtained similar results, and in 1906 Bles published a detailed report of a method by which *Xenopus* could be bred in a small laboratory aquarium. The major points established by Bles (1906) were: (1) The *Xenopus* should be allowed to hibernate over the winter. As a prerequisite for successful hibernation, the toads should have had adequate light, heat and food the previous summer. (2) In the spring, the temperature of the aquarium

[1] This study was supported by a grant from the Committee for Research in Problems of Sex, National Research Council.

should be raised to 22° C. (3) Water should be drawn off daily, allowed to cool, and then run back slowly in such a manner as to simulate rain. (4) When the eggs hatch, food in the form of concentrated flagellate cultures must be added daily. Bles raised his tadpoles through metamorphosis, and raised at least one male to maturity in two years.

In addition to these successful endeavors to raise *Xenopus* in captivity a number of aquarists (Kotthaus, 1933, Senfft, 1939, and Hogjaard, 1940) have reported breeding the African clawed toads and rearing the young. It is evident from the above reports that if optimal environmental conditions are established, *Xenopus* will occasionally breed in captivity. However, the methods used are neither dependable nor practical. If optimal conditions are not created the female toads will not ovulate in captivity (Shapiro, 1936a, 1936b) and this fact is a major premise for their use in the pregnancy test.

Hogben, Charles and Slome (1931) were the first to demonstrate that a gonadotropic hormone would cause *Xenopus* females to ovulate. Shapiro, using a human pregnancy urine extract (1936a) and an anterior pituitary extract (1936b) obtained fertile eggs, and reared some tadpoles, but he was unable to bring them through metamorphosis. Landgrebe and Purser (1941) reported briefly a method of raising *Xenopus* using gonadotropic hormone, but details are lacking, and their method of feeding the tadpoles is cumbersome.

BREEDING METHODS

Sexing the Adults. While it is difficult to sex the immature toads, the sex of the adults can be readily determined. The three cloacal lips of the female are large and are easily distinguished, while the homologous structures on the male are small and are barely visible. Secondly, black nuptial excrescences are usually present on the ventral surfaces of the forelimbs of the males. These pads have been pictured and described in detail by Berk (1939).

Maintaining the Adults. We have found that an adult pair will get along very well in a 10 to 15 gallon aquarium, or 4 to 6 pairs in a 50 gallon tank. If care is taken not to put more food in the tanks than the toads will consume, the water need not be changed more than once a month or even less frequently. To minimize pollution of the water, we have placed aerators in our tanks. While this is a convenience, it is not absolutely necessary. Finely minced beef muscle or liver fed daily is a satisfactory diet. In addition we have found that a coarse grade of dehydrated tropical fish food (consisting of oatmeal, lettuce, spinach, dried shrimp and liver) is a convenient and satisfactory alternative. We do not recommend a diet consisting wholly of the dehydrated food.

The toads can stand considerable and even abrupt changes in temperature, but it is probably best to keep them around 70° C. They seem to thrive under light conditions varying from total darkness (Bellerby, 1938) to bright sunlight. Here again a moderately lighted room is probably most desirable. Landgrebe (1939) studied the effects of food and crowding on the maintenance of reproductive activity in *Xenopus*. He recommends feeding the toads with liver at least once a week, and either keeping the toads in running water, or else at a maximum concentration of one 50 gr. toad per liter of water. Some investigators (Elkan, 1938, Crew, 1939) claim that the water must be changed after each feeding. We have not found this to be necessary. Only large quantities of excess food pollute the water, and this can easily be avoided. We have received inquiries concerning the sudden deaths of large numbers of toads during the summer months, and we too experienced this difficulty on one occasion. Changing the water and sterilizing the tanks did not help, but when the remaining stock was removed to a dark cool room, no more deaths occurred. On the other hand, we have been able to keep part of our stock healthful and in good reproductive condition in a sunlit greenhouse at 25° C., where the temperature some-

times rises to 35° C. Other than the one occasion mentioned above, deaths have been very infrequent.

Induction of Spawning by Hormone Treatment. To induce spawning, the males and females were injected intraperitoneally with a sheep anterior pituitary extract known as 3819-preloban.[2] The doses used and the results obtained are recorded in Table I. If no eggs were obtained with one dose a second injection was given 24 hours later, and if no eggs resulted, a third injection was given 48 hours after the first treatment. Toads that did not respond after the third treatment were not given any more hormone, and are not listed in the table. One mg of preloban is approximately equal to 1.2 rat units.

From Table I it is evident that a single dose of from 4 mg to 8 mg caused the female to ovulate too rapidly resulting in large complements of infertile eggs. (There is also a possibility that the infertility of the eggs was caused by too strong an injection into the male, but this is unlikely.) It is also apparent that a dose of 2 mg was slightly subminimal for most females, and that when this amount of hormone was injected 24 hours after the first treatment many pairs yielded large quantities of fertile eggs. A second dose of 4 mg for the females was also satisfactory. Finally it is evident that two successive injections of 2 mg each were sufficient for the male. It is probable that many of the males did not require any hormone treatment to induce breeding.

Due to the scarcity of adult toads, other gonadotropic hormones have not yet been tested. The studies of Weisman *et al.* (1941, 1942b) show that the dose required to cause a female to ovulate varies considerably for each hormone used. From these data a slightly subminimal dose for each hormone which these authors used can be calculated, and it is reasonable to predict that for the female, a subminimal dose followed 24 hours later by twice that amount should be satisfactory. Likewise, two

[2] The anterior pituitary extract was supplied by the Winthrop Chemical Co., New York.

successive subminimal doses should be satisfactory for the male.

The injected pair should be placed in a 15 gallon aquarium. It is advisable to place a water soaked log on the bottom of the tank, since the female attempts to deposit the somewhat adhesive eggs on solid objects in the water. From the spawning of male 9 and female 10

TABLE I

INJECTIONS OF SHEEP ANTERIOR PITUITARY EXTRACT—PRELOBAN

Pair	Date of first injection	Size of first injection in mg	Size of second injection in mg (24 hours later)	Size of third injection in mg (24 hours later)	Results Within 24 hours after last injection
♂ 23 ♀ 23	10/31/40	8* 4			Large complement of eggs—none fertile
♂ 24 ♀ 31	11/3/40	8 8			Large complement of eggs—none fertile
♂ 5 ♀ 6	12/2/40	3 2	3 2	3 2	Large complement of eggs—many fertile
♂ 13 ♀ 15	2/18/41	2 2	2 2	2 4	Large complement of eggs—app. 75 per cent. fertile
♂ 7 ♀ 8	3/14/41	2 2	2 2		Large complement of eggs—app. 75 per cent. fertile
♂ 11 ♀ 12	3/28/41	2 2	2 2		Large complement of eggs—few fertile
♂ 18 ♀ 19	5/12/41	2 2	2 2		Small complement of eggs—many fertile
♂ 33 ♀ 20	3/4/43	2 2			Small complement of eggs—many fertile
♂ 1 ♀ 14	4/1/43	2 2	2 4		Large complement of eggs—many fertile
♂ 12 ♀ 35	4/1/43	2 2	2 4		Large complement of eggs—many fertile
♂ 9† ♀ 10	5/19/43	2 2	2 2	2 2	Large complement of eggs—app. 10 per cent. fertile

* 1 mg preloban is equal to approximately 1.2 rat units.
† Pair reared in laboratory.

samples were taken of the loose eggs, and also of eggs adhering to the log. These counts showed that only 10 per cent. of the loose eggs were fertile while 75 per cent. of the adhesive eggs were fertile. While in other spawnings the percentage of fertile loose eggs was estimated to be much higher, the use of the log still seems desirable.

The adults should be removed from the tank as soon as possible after the termination of the oviposition to prevent their damaging the eggs.

Rearing the Tadpoles. After the adults have been removed and the eggs have been checked for developing embryos, an ordinary aquarium aerator should be placed in the tank. One or two lettuce leaves previously boiled and cooled should now be added to the water. No attempt should be made to remove undeveloping eggs, since the decaying eggs and decaying lettuce will form the culture medium for bacteria and other microorganisms upon which the tadpoles will soon feed. The outer leaves of the lettuce (generally discarded by the vegetable dealers) are satisfactory. They should be brought to boil in a little water, and may be stored in a refrigerator until needed (Hyman, 1941).

The tadpole aquarium should be checked once every other day. If the lettuce is mostly disintegrated more should be added. If too much lettuce is added the water will begin to develop a distinctly foul odor and the tadpoles will start floating at the surface. When this condition is noticed, the aeration should be increased and no more lettuce should be added until the foul odor disappears. After a few weeks considerable debris will accumulate in the tank, but we have not found this to be the least bit harmful, and it should not be removed. We have observed that *Xenopus* tadpoles are rather sensitive to handling. Consequently such procedures as cleaning the tanks should be avoided if possible. If the proper aeration is maintained and not too much lettuce is added at one time, it is possible to bring all of the tadpoles through metamorphosis without ever changing the water.

The tadpole aquarium may be placed in any situation varying from total darkness to bright sunlight. However, too much direct sunlight will cause the water to turn green, and this will make inspection difficult.

If it is desired to rear the tadpoles rapidly, they should be thinned out. This is done by filling other aquaria with water and adding some boiled lettuce, at least 3 or 4 days before using. Fresh tap water should be avoided. The embryos or young tadpoles are delicate and should

be handled gently. If about 10 to 20 tadpoles are placed in a 15 gallon aquarium, the first tadpoles should begin to metamorphose in one month, and the slowest growing tadpoles should start metamorphosing within two months.

If it is desirable to prevent the tadpoles from metamorphosing rapidly (as for demonstration purposes), they should be crowded (100–200 in a 15 gallon tank). When this is done the fastest growing tadpoles will metamorphose in one to two months, while the slowest growers will remain in the larval stage for 4 to 6 months or more.

Metamorphosis. As contrasted with our local Anura, this stage in *Xenopus* does not present any difficult problems. A variety of small live foods are satisfactory for the baby toads such as tubifex worms, Naid oligochaetes, white worms (*Enchytrae*) and Chironomus larvae. (See Hyman, 1941, for methods of culturing some of these forms.) The oligochaetes were sometimes grown in the water along with the tadpoles.

As the tadpoles complete their metamorphosis, they may be removed to a new tank. After a month or two, live food may be dispensed with, but even before this if live food is not available, a medium grade tropical fish food can be used. This should be sprinkled into the water directly above the little toads to attract their attention. Liver cut into very small pieces should also be used.

The F_1 Generation. In 6 to 8 months some of the males born in our laboratory from wild stock became mature and were successfully mated to our wild females. These males were less than half the size of a full grown adult. The females born in captivity appear to be less likely to reproduce. Although some of the females are over $2\frac{1}{2}$ years old and are almost full grown, only one of them has bred thus far. This particular female was approximately 2 years old when hormone treatment began. It is not clear why the F_1 females are reluctant to breed. They might still be too young. The conditions under which they were raised might not be satisfactory. Treating the animals with gonadotropic hormone before they were

fully mature might have resulted in an inhibition of the ovaries or an anti-hormone effect.

From the single F_1 spawning an F_2 generation is being reared and there is some possibility of developing a strain of *Xenopus* that will readily breed in captivity.

CONCLUDING REMARKS

The question has been asked many times why our local frogs and toads can not be used for pregnancy diagnosis. The comparison of *Xenopus* with local American Anura as given by Weisman, Snyder and Coats (1942b) is not satisfactory. These authors state that "The Xenopus frog differs from the common variety of American frogs in that it constantly ovulates and accordingly carries the ova in its abdomen. The eggs are extruded in natural life only upon stimulation by the male of the species." There is no reason to believe that these toads constantly ovulate. Similar to American Anura, *Xenopus* has a definite breeding season (from July to September—African spring Shapiro, 1936a, Berk, 1938), the only difference being that the *Xenopus* female apparently lays part of the eggs each night for a number of successive nights (Bles, 1906). That the clasping male stimulates the female to ovulate has been hypothesized many times for Anura (Hartman, 1939, quoting Nussbaum, 1895, 1897, Bragg, 1941). However, the evidence for this concept is weak. On the other hand, there is some evidence that in *Rana* and *Xenopus* (Noble and Aronson, 1942, Shapiro, 1936c) the male will not clasp the female until she is first stimulated to ovulate, apparently by environmental changes in rainfall and temperature (Berk, 1938). If the environmental conditions are satisfactory, there is reason to believe that female *Xenopus* would extrude eggs even if isolated from males. It is possible that the call of the males might act as an auditory stimulus, or that the sight of the male might act as a visual stimulus, and that these stimuli might be two out of many which cause a female to ovulate, but this has not as yet

been demonstrated. In our aquaria, the presence of calling males never caused any female to ovulate.

The major differences between *Xenopus* and most other Anura relative to the pregnancy test is the extreme sensitivity of the ripe ovaries of the clawed toads to various mammalian gonadotropic products as compared with the slight sensitivity of other Anura to these same preparations. Secondly, it appears that in *Xenopus,* all the eggs that are ovulated pass promptly down the oviducts and are extruded even though they be few in number. In many other Anura particularly *Rana,* the ovulated eggs accumulate in the lower part of the oviduct (uterus). Whether such eggs are extruded, oviposited (Noble and Aronson, 1942), or retained indefinitely in the uterus depends upon such factors as the number of eggs in the uterus, rate of ovulation, the clasp of the male, temperature, etc.

While it might never become economcially practicable to raise *Xenopus* in captivity either for the pregnancy test, for food, or for any other purposes, if these toads can be bred with reasonable success, the value of *Xenopus* as a laboratory animal will be greatly enhanced. For those not well acquainted with *Xenopus,* the following virtues of this toad as an experimental animal as compared with our local Anura are listed: (1) Live food (or forced feeding) which most other Anura require, is not needed. (2) Any kind of tank or container of water is satisfactory to keep the animals in good health. (3) If necessary, the water need be changed only a few times a year, and feeding may be reduced to once a week. (4) While metamorphosis presents a difficult hazard to the breeder of most Anura it offers no difficulty whatsoever for *Xenopus.* The recently metamorphosed toads can be fed just as easily as the adults.

It is hoped that this report will answer a few of the questions concerning the breeding of *Xenopus,* and will act as a guide to those who may be contemplating such a project.

ADDENDA

(1) A paper on the breeding of *Xenopus* has just been received. (Paul Gasche—1943. *Rev. Suisse de Zool.*, 50: 262–269). The method of raising the tadpoles which this author suggests does not appear to be very appropriate. On the other hand Gasche states that with intensive feeding of liver, and by changing the water on alternate days, mature females were reared in eight months.

(2) Since this report was submitted for publication, four additonal laboratory reared females were induced to ovulate. Each female received a daily injection of 2 mg of preloban for four consecutive days.

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GENETICS OF BODY SIZE AND RELATED CHARACTERS

I. Selecting Small and Large Races of the Laboratory Mouse

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Introduction

An experiment was started in 1939 whose objective was to produce by selections from one original population both an extremely small and an extremely large-bodied race of the laboratory house mouse. Such races did not exist either in the rat or mouse, though Goodale had very successfully increased mouse weights by selection, and considerable size differences are found between closely allied species in mice (*Mus bactrianus* and *M. musculus*), and in guinea pigs, and between some breeds in rabbits.

This work was undertaken in the belief that stocks showing the widest possible contrast in body size, once developed in some common small experimental mammal, might prove as useful aids in genetic studies of typical quantitative characters, as have the stocks possessing diverse color combinations for the study of qualitative characters. They could be used advantageously for demonstrations and for much-needed more exact investigations on inheritance of differences in size, in growth rates and in hybrid vigor. Meanwhile the trends of the selections themselves throw some light on the nature of size inheritance, and may aid in reinterpreting some older selection experiments.

It will be noted also (in Part II) that, clustering around body size as the central character, are found associated intimately a whole system of other correlated or satellite characters of not a little general biological interest, involving as they do such basic problems as the physiology of mammalian reproduction, fertility (litter size), be-

havior (activity and wildness), growth and relative growth (ratio of appendage length to body measurements).

The research was done with the laboratory mouse, but the findings quite evidently have a more general significance, for they apply, in some respects at least, as has been shown (MacArthur, 1942), to man and to other mammals, both domesticated and wild. And, as it turns out, the production of these races parallels in a number of ways raciation processes occurring in nature.

The results certainly illustrate the great efficacy of selection as an agent for the modification of size (and the various characters that follow in its wake) and are likely, of course, to have some practical bearings in the way of offering suggestions or giving directions for breeding more economical, larger and more prolific farm animals.

Plan of the Experiment

Preparation of the Foundation Stocks. The population from which the selections began was derived ultimately from several fixed inbred strains of laboratory mice, commonly used in many laboratories for demonstrations of color inheritance. Seven strains in all were interbred in four separate crosses, and their hybrids were again interbred at random to produce a large "F_2" population, the foundation stock.

The strains used were the familiar long-inbred "dilute brown" (*dba*) and "silver chocolate" (*sv ba*) from the Jackson Memorial Laboratory; "black and tan" (a^t), and pink-eyed, short-eared "dilute brown" (*ps dba*) from the University of Michigan; and our own stocks of "albino" (*c*), "cinnamon spotted" (*bs*), and "agouti."

These strains were chosen principally because they were average and undistinguished in body size, carried no unusual mutant size genes (for dwarfness, etc.), and were free from abnormal factors (tailless, hairless, lethal yellow, high cancer, etc.). They were hybridized to increase the field of variation, by bringing together into

the melting pot as wide an assortment as possible both of the more normal qualitative Mendelian factors and also, and especially, of the size genes or modifiers controlling quantitative differences in growth rates and body sizes.

Four different matings produced young as follows:

Parents		F_1 *young*
female	male	
black and tan	cinnamon spotted	9
albino	dilute brown	3
silver chocolate	ps^{cd}ba	6
black and tan	agouti	8

The 26 F_1 young were allowed to interbreed uncontrolled in a single large cage. They produced a large F_2 population ultimately numbering several hundred mice.

Separation of Reserve and Experimental Stocks. The month-old young were removed from the F_1 breeding cage and isolated by sexes in two large cages. These were later divided at random into an experimental portion from which the special size selections were made, and a remnant reserved as control. The larger control lot is being maintained as a single group by mass breeding without deliberate selection.

The experimental group withdrawn consisted of equal numbers of females and males. As they matured 30 were mated without regard to size, each pair in its own small cage. Their first and second litters, totaling 101 males and 117 females, were reared to at least 60 days. These "F_3" litters collectively constitute Generation 0 of the selection experiment.

Establishment of Small (S) and Large (L) Lines. The young of each litter were weighed collectively at birth, and later sexed, phenotyped and ear-marked and weighed again at least at 30 and 60 days. The weight approximately doubles between 30 and 120 days, but at two months of age the growth curve has so flattened out that the 60-day weight affords a reliable indicator of the final body weight.

Each litter was then roughly classified as either

"small" or "large," judging from the average of weights of its component individuals. Small mice and especially those from half of the litters with smaller-than-average 60-day weights became the progenitors of the small race. The successive generations selected to maintain and build up the small line are recorded in this paper as S_1, S_2, . . . S_8. The large line (L) similarly began with the large individuals taken from the litters of higher-than-average 60-day weights. The small and large lines have since remained isolated and self-sufficient. No genes have been exchanged between them, since they were first separated, and no new blood has been introduced from outside into either line.

That the preliminary mixing in the melting pot had been reasonably efficient (though of course far from complete) is shown by the fact that all the different qualitative genes represented in the original strains were recognized, at least in early generations, in both the S and L lines, and they still remain in the remnant "control" stock cage.

Care of the Stocks

To reduce environmental inequalities to a minimum all the mice were kept continuously in the same air-conditioned room-temperature laboratory murarium, fed the same foods (wheat grain, fox chow, lettuce, etc.) and the cages were rotated in position from time to time. Seasonal changes in daylight, artificial lighting, humidity and temperature were shared by all groups alike. No artificial stimulation, by feeding of vitamin concentrates, like wheat germs, or by hormone treatments, was employed on any stocks. The changes produced by selection in the body sizes and other characters are thus unquestionably, for the most part, of a permanent and hereditary, and not of a temporary and environmental nature.

System of Mating in the S and L Lines

The small and large selection lines are maintained by the mating each generation of approximately 5–10 males

and 20–35 females. These are always bred as pedigreed pairs, never en masse. In general each male is mated at one time or another with 4 to 8 or 10 females in his breeding cage, from which each pregnant female is removed to her special numbered cage (*e.g.*, 1). Here her young are born and reared until weaned; the young females are left with the mother, while the young males are transferred to their correspondingly numbered cage (1 a). The mother may then be remated with the same male, or (if the first litter was not promising at the 30-day weighing) with a different male, or even discarded.

In general the attempt is made to secure about 10 progeny from each pairing from which to judge the genetic worth of the family. For this purpose one litter usually suffices in our present L_8 stock, but two successive litters are required in the S_8 stock, owing to the small average number of young born in each litter (part II).

The S and L stocks are maintained with little continuous inbreeding and no deliberate line breeding. No separate "sublines" or "families" are established in either stock. The S stock, for instance, contitutes at any generation one interbreeding population, in which new combinations of size genes are brought together by diverse pairings, and the best combinations are detected, if possible, and preserved by selection. On the other hand, inbreeding is not avoided, for sibs and other relatives are often mated. The intention is to mate assortatively, like with like, more or less irrespective of relationship, and also without attempting to fix (make homozygous) any gene pairs, or, if they chance to be fixed, to keep them so.

Since each breeding population is relatively small, consisting as it does of what is something like a "5–10 sire herd," closed to outside blood, one would expect a slow loss of heterozygosis, and loss or fixation each generation of some size genes and some qualitative genes. From Wright's formula the rate of reduction of the remaining heterozygosis may be roughly estimated as $\frac{1}{8M}+\frac{1}{8F}$, or

something like 1 to 3 per cent, per generation. This should gradually cause the lines both to change gradually by "drift" and to become more uniform, even independently of selection.

Criteria of Selection

In each of the lines the "best" 5–10 males and a select group of some 20–35 females have to be picked as breeders to produce the following generation. These are commonly chosen from about 60–120 individuals of each sex, in each line. In general we wish to identify, save and breed the group that would represent a deviation from the average of about $1.3-1.6\,\sigma$ for males, and $0.7-1.0\,\sigma$ for females.

In the L line, for example, the object is to accumulate and concentrate all possible different and possibly interacting large size genes by the plus selection. The mice most likely to possess such genes are chosen on the basis of:

(1) *The progeny test.* The principle of the progeny test is given in the familiar quotation: "By their fruits ye shall know them." Ten or twelve young afford a fair practical sampling of the genotype of the parents. Sixty-day weight data are at once transferred from cards to summaries, tabulated as a frequency table, so that the body size of each sibship and individual may be compared with the frequency distribution of the preceding and current generations. The already highly selected and extreme parental pairs are not usually even equalled by their progeny. Running progeny tests discover which few of the select pairs actually do transmit exceptional size. If all or nearly all the progeny excel the average of the preceding generation in 60-day weights the parents must have formed a favorable combination as partners.

(2) *Individual performance.* Those individuals which on their own records of growth in size appear to represent a promising combination of the various size genes segregating and assorting within a sibship of 10 or 12

are favored tentatively because they have exceeded their sibs.

(3) The selection is thus based primarily on the progeny tests and individual phenotypes, with a further effort to keep the basis of selection broad by choosing for breeding at least one female from each even moderately promising family, rather than achieving a more spectacular but less lasting success at a sacrifice of continued variability.

Selection between breeding pairs proceeds actively within a given generation. Progeny distinctly below standard at 30 or 60 days are discarded, and the parents may also be culled or mated to different partners. The pairs which have distinguished and proven themselves by producing a litter especially promising at 30 or 60 days are again mated together or each with other promising mates. These additional litters are evidently particularly important features of successful progress in selections guided by progeny tests. (An incidental result of this practise is that the mean of the population is artificially shifted and the distribution skewed toward the small end of the curve in the S stock and toward the large end in the L stock.)

A certain amount of reproductive selection of course occurs each generation against barren matings and shy or late breeding individuals. Extremely obese large mice or runty "dwarf" small ones have usually proven sterile or practically so. This is fortunate, since we did not aspire to produce freak types. On the whole the few monstrous or "abnormal" or sickly mice that appear present no major problem, since these seem to be practically self-eliminating.

A further point should be mentioned. The largest stock females may produce as many as 16 or 18 living young in one litter. Since so many young might be handicapped in their growth before weaning the number of young retained in such litters has (since the 8th generations) been restricted to 10.

Progress of the Plus and Minus Selections

Gradual and Progressive Divergence of the Small and Large Races. Selection, especially when it follows recent extensive hybridization, is a powerful agency for change in organisms. There were special reasons for expecting success with this material. Recombination of factors in

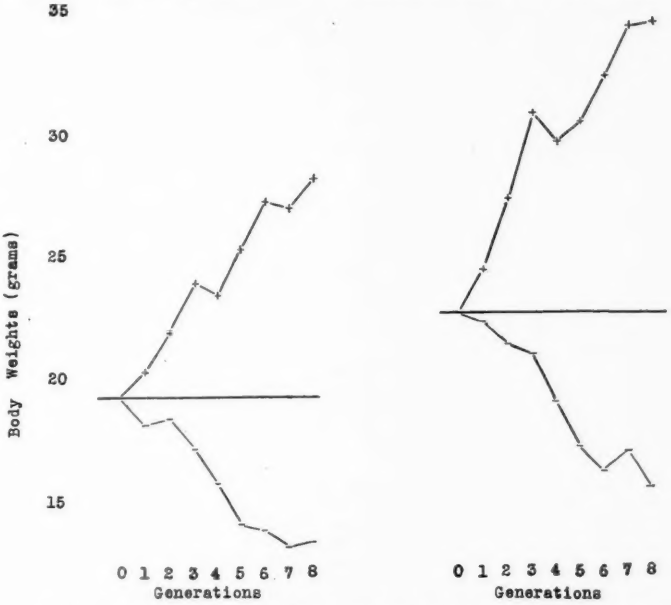

Fig. 1. Divergence of mean 60-day weights of females (left) and males (right) during 8 generations of plus selection (— + — +) and minus selection (— - — -).

mouse chromosomes is known to be comparatively high and to provide a continual supply of new variability by crossing-over (Crew and Koller). Goodale found it rather easy to increase size in an albino stock.

Actually the mice used proved to be so plastic and variable that they are being rapidly "made over to size" by the plus and minus selections. Regression is generally positive, large parents typically producing large progeny, and small parents small. As a general rule

each succeeding generation sees the small and large races diverging more and more in their average 60-day weights (Fig. 1), and still more in their final weights. Moreover, there is every prospect of continued, but undoubtedly slower, divergence with further selection.

The size difference between races is already present and distinct at birth and steadily widens with growth, at first quickly and then slowly, in the postnatal period. When neutralized as to sex the difference, 26 per cent. (1.18 *vs.* 1.49 gms.) in new-born, birth-fed young, increases to 68 per cent. (11.74 *vs.* 19.76 gms.) at 30 days, and 108 per cent. (14.94 *vs.* 31.11 gms.) at 60 days, and

TABLE 1

LEAST, AVERAGE AND GREATEST 60-DAY WEIGHTS OF MALE AND FEMALE MICE THROUGH THE FIRST EIGHT GENERATIONS OF SELECTION TO DECREASE SIZE (SMALL RACE, LEFT) AND TO INCREASE SIZE (LARGE RACE, RIGHT). THE FEW RECESSIVE-TYPE "RUNTS" EXCLUDED. EACH RANGE AND AVERAGE BASED ON 59–115 INDIVIDUALS.

Generation	Small line						Large line					
	Males			Females			Males			Females		
0	18.5	23.2	31.9	13.9	19.5	26.9	18.5	23.2	31.9	13.9	19.5	26.9
1	16.6	22.9	28.6	12.9	18.6	23.5	18.2	24.6	32.3	15.1	20.8	27.8
2	15.5	21.9	27.0	13.3	18.8	21.9	20.1	27.9	36.3	14.6	22.2	30.2
3	14.7	21.4	29.5	13.0	17.6	23.0	21.1	31.2	43.0	18.6	24.4	38.4
4	12.7	19.5	29.0	10.7	16.1	22.8	23.4	30.0	36.9	18.2	23.9	36.4
5	12.6	17.9	22.8	10.8	15.2	19.7	22.9	30.9	41.4	19.6	25.6	36.8
6	14.3	18.1	24.4	11.9	15.3	21.0	24.6	32.7	41.6	20.6	27.6	36.2
7	10.4	17.4	22.0	9.7	13.9	18.0	28.2	34.7	42.6	19.8	27.5	37.3
8	10.8	16.0	20.6	10.6	13.8	22.6	27.5	33.7	41.0	18.7	28.4	38.2

is still greater at advanced ages, especially in females. Growth is more rapid and more prolonged in the large race.

For a period during the fifth to the seventh generations a small number of runts (11 out of 212 males and 15 of 239 females) segregated as recessives from the small line only. Somatically they resemble the dwarf mutant. As their growth practically ceases at about 7 to 9 grams, they form a separate group of distinctly smaller size with short ears; they are of low vitality and apparently sterile. Selection against pairs producing them has since all but eradicated this type.

Quantitatively the trends of the changes induced by selection consist in a general, but not entirely steady,

decrease of the means and of both extreme weights in the small race, and their increase in the large race (Table 1).

In Fig. 2 are shown the frequency distributions of male 60-day weights in the unselected population, and in the small and large lines after four, and again after eight generations of selection. It was noted above that the

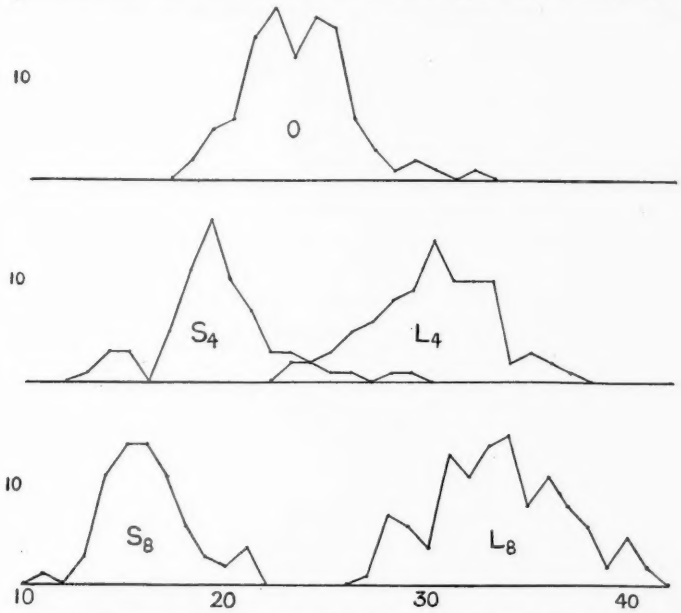

FIG. 2. Frequency distributions of 60-day weights of male house mice in the foundation stock sample (0, above), after 4 generations of selection (middle row, S_4 left, L_4 right), and after 8 generations of selection (lower row, S_8 left, L_8 right).

distributions are likely to be skewed arbitrarily by rematings. Large sibships of similar sibs tend to make the numerous lesser peaks on the curves.

After the sixth generation, overlapping of the S and L curves (each based on samples of less than 116 mice) became rare and has now practically ceased. The least mice of the large race are larger than the greatest of the small race.

TABLE 2

DIFFERENCES BETWEEN AVERAGE 60-DAY WEIGHTS (GRAMS) OF THE LARGE AND SMALL RACES OF MICE RESULTING FROM EIGHT SUCCESSIVE GENERATIONS OF PLUS AND MINUS SELECTION. THE GAP BETWEEN THE RACES HAS WIDENED STEADILY BUT BY LESSENING AMOUNTS AS THE EXPERIMENT PROCEEDS. FOR MALES, FOR INSTANCE, THE DIVERGENCE PER GENERATION AVERAGED 2.7 GRAMS IN GENERATIONS 1–4; 1.8 GRAMS IN GENERATIONS 5–8; AND LESS THAN 1 GRAM IN THE LATEST GENERATIONS, 9–12.

Generation	Differences between races	
	Females	Males
0	0	0
1	2.25	1.69
2	3.31	5.99
3	6.73	9.76
4	7.76	10.51
5	10.44	13.07
6	12.30	14.78
7	13.95	17.44
8	14.79	17.81

There are, of course, far smaller mice in the small race (S_8) and far larger ones in the large race (L_8) than were found among the approximately equal numbers reared in the initial generation before selection began.

After 8 generations of selection the average difference between the races clearly exceeds the mean absolute weight of the small race (Table 2). Small race males then averaged 16 grams; the large race males were 17.8 grams heavier at the same age and generation. The small females had a mean weight of 13.8 grams, while large race females exceeded this mark by 14.8 grams.

Data and calculations condensed in Table 3 show the significance of the size differences brought about by selection and also the promise of continued success with

TABLE 3

SIZES (60-DAY TOTAL WEIGHTS IN GRAMS) OF MALES AND FEMALES FROM THE UNSELECTED FOUNDATION STOCK (GEN. O), AND FROM THE SMALL AND LARGE RACES AFTER FOUR AND EIGHT GENERATIONS OF MINUS OR PLUS SELECTION. THE DIFFERENCES BETWEEN MEAN WEIGHTS OF S_8 AND L_8 MICE EXCEED 28 STANDARD ERRORS.

Generation	Males			Females		
	Mean weight	Standard deviation	Coefficient of variability	Mean weight	Standard deviation	Coefficient of variability
L_8	33.71 ± 0.31	3.31	9.81	28.43 ± 0.39	3.83	13.48
L_4	30.00 ± 0.30	2.76	9.20	23.87 ± 0.34	3.52	14.72
O	23.16 ± 0.26	2.56	11.01	19.51 ± 0.25	2.65	13.52
S_4	19.49 ± 0.32	2.62	13.44	16.11 ± 0.26	2.21	13.72
S_8	16.14 ± 0.25	2.11	13.07	13.80 ± 0.19	1.83	13.25

further selection. Over the eight generations there has been no very definite diminution of total variability in 60-day weights, as measured by the coefficients of variability. But recent gains (and losses) in weight by selection appear to be falling off slowly (see data in legend of Table 2).

Interpretation of the Results of the Selections

It is desirable to establish that at least approximately equal selection efforts were made to reduce size in the S race by accumulating minus size genes, and to increase size in the L race by discovering and saving large-size genes. During the eight generations of breeding the actual selections in the small race were based mainly on data obtained from 166 different fertile pairings and from 230 litters of progeny. The selections meanwhile made in the large race were based on 144 different successful pairings and on 213 litters of young. There were thus in fact more pairings made, more cages occupied and more litters produced and used in the "small" than in the "large" selections. But it is only fair to note, on the other side, that, owing to the gradual, and at first unsuspected, divergence of litter sizes (number of young per litter) in the two races (see part II) the actual number of young produced and reared to the final weighing ages was greater in the large race than in the small. In all, 1,099 mice of the small race and 1,504 of the large race were weighed in at 60 days. These were divided about equally between the sexes (1,291 males to 1,312 females).

Selections were based primarily on litter or sib averages plus uniformity, secondarily on individual phenotypes. As seen above, there was a slightly better chance of picking exceptional litters in the small race, exceptional individuals in the large. Selection was rather more intense between litters in small, within litters in large. Whether these factors approximately balance we do not know. At any rate it was the intention to try

equally hard throughout the experiment to change the sizes in both sexes and in both directions.

Plus selection more effective than minus selection. Though about the same efforts were expended to raise as to lower body size, it is clear that divergence from the original stock was not equally rapid or marked in the two directions, providing the divergence is measured in absolute grams of body weight gained or lost during the selections (Fig. 1 and Table 4).

It is apparent from these data (1) that the large race has increased considerably more and faster by plus selection than the small race has decreased in size by minus selection, and (2) that the size of the male mice has changed more than the size of females in both plus and minus directions. Thus the males gained 10.55 grams and lost 7.02 grams, while the females gained only 8.92 grams and lost but 5.71 grams.

TABLE 4

AVERAGE 60-DAY WEIGHTS IN GRAMS OF MALE AND FEMALE MICE OF THE FOUNDATION STOCK (O) AND AFTER EIGHT GENERATIONS OF PLUS SELECTION IN THE LARGE RACE (L_8) AND MINUS SELECTION IN THE SMALL RACE (S_8). GAINS AND LOSSES EXPRESSED IN ABSOLUTE GRAMS, IN DIFFERENCE OF LOGARITHMS OF WEIGHTS, AND IN PERCENTAGES ($O > S_8$, $L_8 > O$).

	Males				Females			
		Difference in				Difference in		
	Weight	Grams	Logs	Per cent.	Weight	Grams	Logs	Per cent.
Large race (L_8)	33.71				28.43			
		+10.55	.1629	45.6		+8.92	.1633	45.7
Foundation (O)	23.16				19.51			
		−7.02	.1569	43.5		−5.71	.1503	41.4
Small race (S_8)	16.14				13.80			

Inadequacy of the current theory of size inheritance. The orthodox theory, illustrated in most genetics texts by inheritance of length of corn ears or tobacco corolla tubes, implies that plus and minus size genes or modifiers exert effects, which may be stated as precise quantities, and each of the multiple factors controlling size is considered to add to or subtract from the body weight some

constant and definite amount measurable in ordinary units such as grams. Our results are not in accord with this arithmetic view. The data cited count rather heavily against it, and in fact raise considerable doubt as to the perfect symmetry of the "normal" curve of distribution of size differences.

The geometric theory of size gene interaction. On the other hand, the gains by plus selection and the losses by minus selection become nearly, though not quite, equal when the changes in either direction are measured and expressed in the form of difference of logarithms or in percentages (Table 4).

When similarly expressed the average male 60-day weight exceeded that of females by 18.7 per cent. in the unselected stock, and by substantially the same percentage in both the small race (17.0 per cent.) and the large race (18.6 per cent.). Calculated as percentages the gains or losses are practically balanced and equal in both sexes, and the sexual dimorphism in size remains at least approximately constant throughout the experiment. Here again the effects of the selections on growth rates and sizes are most simply described by transforming the data to a logarithmic or percentage scale.

These basic data clearly suggest that the influence of a size gene or modifier is not a constant quantity definable in absolute amount, but rather that the effect of such a size gene or modifier increases as body size increases and lessens as body size diminishes. In other words, the characteristic and essential effect of a size gene is that it depends on some standard of reference and is relative, being proportional to body size as potentially determined by the remainder of the genotype. Size genes appear to multiply rather than summate each other's effects. This is the same geometric concept of size gene action and interaction in inheritance that was arrived at by previous studies of fruit sizes in hybrids from crosses of large and small-fruited varieties of tomatoes (MacArthur, 1941). The fruit sizes from dozens of crosses of different types

fit too closely and too regularly to the geometric mean to be plausibly confused, as Mather contends, by phenomena of dominance and epistasis.

On this geometric view selection effects are comparable to growth changes. At least over a limited series of generations, while plus or minus genes are abundant and readily accumulated and concentrated, the rate of change by selection may be likened to rate of growth of individuals or populations in time, and expressed as an exponential increase of a capital by the compound interest rule. Thus the rate of change by selections is most conveniently and adequately expressed, not by the absolute number of grams gained or lost, but by the percentage of gain or loss per generation, the increase or decrease being preferably compounded (at a diminishing rate, of course, as variability declines) each generation.

SUMMARY

A large population was made up by hybridizations of several common laboratory strains of mice which were in no way remarkable as to their body size. From one segregating foundation stock eight successive generations of selections for body size have produced: (1) a race of small body size; and (2) an exceptionally large race, whose individuals average a fourth larger at birth and twice the weight of the small race at 60 days of age.

Size was judged from the 60-day weight, and selections were based on progeny tests, sib likeness and individual phenotype.

The races diverge each generation, but at a lessening rate. Their size distributions show little or no overlap, and the present difference between the races exceeds the mean weight of the small race.

Less grams of body weight were lost by the minus selections than were gained by equal effort at plus selection; and less grams were gained or lost by females than by males.

When measurements are transformed to a percentage or a logarithmic scale the weight gains and losses become approximately equal in both sexes and races.

These data agree with the view that size genes or modifiers multiply each other's effects (that is, act geometrically), and do not simply add to or subtract from the body weight some definite and constant number of grams.

THE DOMAIN OF ETHNOBIOLOGY[1]

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SCATTERED data bearing on the utilization of plant and animal life by primitive peoples in the New World have been accumulating ever since the first voyage of Columbus, but the first attempt to present a systematic statement of the subject appears to be that of Palmer (1870), who published a paper, the content of which was enlarged in a second treatise (Palmer, 1878).

As early as 1875, Powers (1873–74) made an attempt to delimit the field when, in an article entitled "Aboriginal Botany," he employed the term to embrace "all the forms of the vegetable world which the aborigines used for medicine, food, textile fabrics, ornaments, etc." These papers by Palmer and Powers stimulated a considerable amount of interest, as indicated by the appearance of a succession of papers by such men as Powers (1877), Matthews (1882, 1886), Havard (1884, 1895, 1896), Newberry (1887), Coville (1892, 1897), Bourke (1895), Fewkes (1896), Hough (1897, 1898), Barrows (1900), Jenks (1900), Chestnut (1902) and Cushing (1920). Two additional factors contributed materially toward the creation of a deep interest in the aboriginal utilization of plant and animal products. The first was the botanical surveys made in the western United States by representatives of the Federal Government, which brought men into contact with primitive peoples and their methods of exploiting local flora and fauna. The second factor was an exhibit at the World's Fair in 1893 of archeological specimens, such as objects of food, dress, household utensils, etc., from Mancos Canyon, Colorado. At the close of the fair, the University of Pennsylvania secured the loan of this collection.

[1] The author is greatly indebted to Dr. Leslie Spier for carefully reading and criticizing the manuscript.

The Mancos Canyon collection was studied by Harshberger, of the department of botany at the University of Pennsylvania, who described the objects of vegetable origin; and on the basis of this he delivered a lecture before the University Archaeological Society in 1895, publishing the lecture in the following year (Harshberger, 1896). This apparently represents the earliest application of the term "ethnobotany," which now has come into universal use in America. Schiemann (1932) has summarized some of the related ethnobiological material for the Old World, but neither the term ethnobotany nor any cognate term appears to have been employed in the eastern hemisphere. Although Harshberger indicated several directions in which ethnobotany could be of service, he made no specific attempt to define the term, evidently visualizing it in this and other papers as merely the use of plants by aboriginal peoples. About fifteen years after the publication of Harshberger's first paper, Robbins *et al.* (1916) expanded this concept, pointing out that ethnobotany consisted of far more than collecting, identifying and ascertaining the uses of plants by primitive peoples; that it must strike more deeply into the thought and life of the peoples studied, gaining from them their impressions of total environment involving such matters as primitive concepts of plant life; the effect of a given plant environment on the lives, customs, thoughts and everyday practical affairs of the people studied; and the extent of a people's knowledge of plant parts, functions and activities.

Specific studies on the aboriginal utilization of animal life have been less numerous than those on the plant side, and the data relating to the former have not been so well assembled and organized as have those on plants. Henderson and Harrington (1914) published a paper in which they employed the term ethnozoology as coordinate with the older name ethnobotany. Although this work seems to have stimulated little research into primitive animal utilization as such, a very considerable

body of information bearing on the use of animals by primitive peoples is to be found in a variety of publications, which have not been written as special treatises on ethnozoology as, for example, papers by Merriam (1905, 1926), Steensby (1917), Birket-Smith (1929, 1936), Allen (1876) and Hornaday (1889).

For many years, ethnobiological information has been gathered by men with little or no training in the study of the primitive utilization of plant and animal life, and in a manner only incidental to their major fields of investigation with no thought of pursuing such studies for their own sake. The data of ethnobiology, therefore, are scattered in the publications of a wide variety of fields. More recently, a few scholars have adopted ethnobiology as their principal field of interest, and have devoted sustained attention to such studies; as a result we now have in the New World a few professional ethnobiologists, and the field is rapidly taking on the earmarks of an independent line of inquiry, as indicated, among other things, by the recognition which *Biological Abstracts* has given to it within the last few years.

A very large proportion of the ethnobiological investigation in the western hemisphere has been carried on with no reference whatever to a motivating point of view. In the past, and to some extent at present, many data bearing on the utilization of plant and animal life by primitive peoples have been gathered by anthropologists, who obviously have not had the biological background necessary to identify precisely, or even see any necessity for identifying, the organisms in question. This situation often has rendered their data of little value to the biologists. On the other hand, ethnobiological data have just as frequently been gathered by biologists who, completely lacking anthropological training, have, during the course of their investigations, secured no data relative to the importance of these organisms in tribal economy, their manner of utilization, etc. Their publications, constitut-

ing little more than a technical list of organisms used by a given people, are dry and barren, and are almost entirely without significance to any one but the botanist.

In view of the above situation, the ethnobiologist is constantly confronted with the problem of delimiting his attitude toward his field of investigation, of defining his point of view or "center of gravity." This, in addition to the rapid progress made in ethnobiology during the last decade, and the recent emergence of the subject as a rather distinct field of inquiry, have motivated several investigators to attempt to circumscribe and define the field of ethnobiology, more particularly ethnobotany.

Vestal and Schultes (1939) have employed the term *economic botany* "to include all plants or plant products of use to man," stating that, in this sense, economic botany is more inclusive than ethnobotany, "a term by which most investigators have designated the plants used by aboriginal peoples." They have adopted what they regard as the broader term, economic botany, "because not all the uses of plants by primitive people are of necessity aboriginal in origin." More recently, Schultes (1941) has reiterated this point of view, stating that the men of science of one or another school are agreeing rather unanimously to regard ethnobotany as "the study of the relations which exist between man and his ambient vegetation." It would seem, however, that Schultes has stated a situation which does not exist in fact, for there is by no means universal agreement with the point of view as set forth by Vestal and Schultes. Economic botany, to be sure, does at times inquire into the relations between primitive man and plants, but rarely does it do so because of any interest in such primitive relationships for their own sake. The major interest of economic botany is in the relationships between plants and modern or contemporary civilizations, and its only interest in primitive relationships consists in the illumination which they may throw upon the modern scene. Ethnobotany, on the other hand, is concerned primarily with the interrelationships

between *primitive* man and plants. Moreover, ethnobotany is sharply differeniated from economic botany in that it is vitally concerned with the fundamental *cultural* aspects of plant utilization, while economic botany practically ignores the cultural except in a very general way. One need only to consult any text-book or reference book on economic botany to realize that its major interest consists in treating the importance of plants and the nature of plant products in relation to modern man; in exploring the uses to which plants are put as, for example, foods, oils, waxes, fibers, rubbers, resins, etc., including the study of the morphology of plant parts which produce such structures. Therefore, ethnobiology is to be looked upon as a field of investigation which exists in its own right rather than as a branch of economic botany.

Recently Jones (1941) published an excellent article entitled "The Nature and Status of Ethnobotany" which is noteworthy for two definite contributons; (1) clearly defining and delimiting ethnobotany; (2) indicating how ethnobotany touches related disciplines and discussing the problems which have arisen from such contacts. He defines ethnobotany as "the study of the interrelations of primitive man and plants," and emphasizes that it deals exclusively with the interrelations of *primitive* man and plants and "takes for its sole aim the illumination of this contact." The author fully endorses Jones's definition, which, in addition to circumscribing the field of ethnobotany, inherently demarcates it from economic botany.

Neither Jones's paper nor any other purporting to define ethnobotany has presented a point which seems vital in delimiting the field. This is the question as to what shall be the point of view, the "center of gravity," of ethnobotanists toward the subject-matter of their own field. Ethnobiology[2] has many facets and abuts on a number of disciplines, but for the most part it may best be

[2] From this point on the discussion will bear on ethnobiology rather than on ethnobotany, for the comments apply equally well to both plant and animal life.

regarded as marginal to, or as straddling, both biological science and anthropology. It utilizes the data and the methods of both fields. The question therefore naturally arises as to whether this "center of gravity" shall be biological or cultural or a combination of the two. The author does not look upon ethnobiology as a distinct science and discipline, but rather as a sphere of investigation in which scholars, trained in both biology and anthropology, can render a service to the two fields which neither biologist nor anthropologist as such would be competent to contribute.

There exist certain purely biological aspects to ethnobiology, such as the identification of organisms and their parts as employed by primitive peoples. Often, as in the identification of plant and animal archeological specimens, the situation calls for special and ingenious techniques, sometimes highly refined. Despite the biological aspects of this problem, which involves a knowledge of such things as plant and animal anatomy and geographical distribution of organisms, an anthropological background also is very useful in making such specific determinations of ethnographic or archeologic specimens. Thus, knowledge of the people among whom a particular specimen was collected, the physiography of their range, their food habits, relations with other peoples, etc., are of considerable aid in identification, even if they consist only in eliminating certain organisms from consideration.

When the average person knows that a specific plant or animal was used by a given primitive people, he is by no means content to stop there. It is a thoroughly human quality to inquire what the organism was used for, precisely how it was utilized, the antiquity of its utilization and what the people who employed it were like. When a plant is seen growing in nature, knowing its name may go a long way, if not the entire way, toward satisfying the average person's interest in it. But when it is found in association with coal, a peat bog, an archeological ruin, a Papago storehouse or poisoning fish, it assumes an

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as knowing which ones they do employ. For example: There is no logical reason why any available food should have been completely excluded from a people's diet. Yet, some of the Northwest Coast tribes, such as the Kwakiutl on Vancouver Island, would not touch venison, even though deer were abundant in their territory. Spier (1930) has pointed out that the Klamath Indians' leaning toward fish as their staple and their relatively small use of deer, elk, antelope and waterfowl, all of which abound in this territory, are arbitrary and not dictated by geographic conditions; and that their food habits are such primarily because they share the habits of the Columbia-Snake river tribes as a whole. That abundance in itself does not guarantee utilization, or at least extensive utilization, is seen again among the Klamath. In addition to the bountiful supply of game in their territory, these Indians had available in Klamath Marsh, according to Coville (1904), the seeds of about ten thousand acres of wokas or the great yellow water lily (*Nymphaea polysepala*), only a small fraction of which was utilized. Despite this plenitude of food, travelers among the Klamath in the early nineteenth century frequently referred to the fact that in winter these Indians were often on the verge of starvation. This was not due to improvidence. For example, the period during which wokas seed could be harvested lasted only a very few weeks, and the process of seed preparation was slow and tedious. This, coupled with the difficulty of transporting the seeds, rendered it impossible to gather, prepare and store large quantities of wokas. Moreover, it is no easier to undertsand why a particular food should be exploited to the wide exclusion of all others, even though abundant, as was the acorn in much of California.

A study of the utilization of specific plants or animals by certain primitive peoples not only shows correlations between culture and terrain, but sometimes furnishes valuable clues to historical circumstances. It may not only throw light on the diffusion of certain plants but on

the histroical diffusion of culture in general. This is nicely illustrated among the Crow Indians of southeastern Montana.

The Blackfoot and the Sarci of the northwestern Plains are the only tribes which share with the Crow the ceremonial planting of tobacco, and this represents the only agricultural effort of these typical bison hunters. Lowie (1920), in discussing the Tobacco Society of the Crow, points out that, as an *organization,* this society is an independent structure, presumably of indigenous growth among the Crow, even though some of its elements have their origin among other tribes. Despite the fact that the elaborate organization of the Crow Tobacco Society is without parallel among the Blackfoot and Sarci, the resemblances between the planting performances of the three tribes suffice to establish ultimate community of origin for the practise. The question naturally arises as to whether the Crow borrowed from the Blackfoot (and the marginal Sarci) or *vice versa.* For the reasons which follow, Lowie concludes that the Crow derived their tobacco agricultural technique from the Hidatsa (living on the upper Missouri), elaborated it ceremonially, and subsequently transmitted it to the Blackfoot, who in turn passed it on to the Sarci together with some of the ceremonial patterns which the Blackfoot themselves evolved: (1) the much more important place which tobacco planting holds among the Crow than among the Blackfoot; (2) the Crow were intimately related to the Hidatsa, who planted tobacco, as well as corn, beans, pumpkins and sunflowers, while the Blackfoot were not in contact with any group from whom they could be expected to have derived the technique; (3) the only Siouan language in which the Crow stem for "tobacco" appears is Hidatsa (*ôpe*). Anthropologists believe there is considerable evidence that the Crow and the Hidatsa were one people not so very long ago, one important consideration being the close similarity of language. Reasons nos. 2 and 3, as presented, shed some light on the

historical diffusion of a cultivated plant, of a culture trait and even of the movement of a people itself; that the Hidatsa share with the Crow a common term for tobacco, as well as an agricultural technique, provides bits of evidence in support of the thesis that these two peoples formerly were one. The fact that the Hidatsa cultivated *Nicotiana quadrivalvis,* in non-ritualistic fashion, while the Crow grew *N. multivalvis,* and only ceremonially, does not vitiate the conclusion that the Crow borrowed their tobacco-growing technique from the Hidatsa and elaborated it ceremonially.

Out of the author's experience in ethnobiology have come certain definite conclusions regarding factors to be kept in mind in carrying on research in this field. These apply particularly to the study of wild plants and animals, although some of them are equally applicable to the investigation of domesticated forms. The student of cultivated plants and animals and of primitive agriculture is advised to consult several of the better treatises on these subjects before undertaking field studies relating to them.

(1) Identification, in exact taxonomic terms, of the plant or animal used. This is less easy with animals than with plants because of the difficulty of obtaining and preserving specimens. In any case, if the investigator is himself not competent to make the exact identifications, he should seek the assistance of specialists.

(2) Relative abundance and availability of the organism.

(3) Indian name for the plant or animal. This often gives an insight into the tribe's conception and knowledge of the structure, function and method of classification of organisms, as well as into related aspects of the people's ceremonial life. Moreover, this name is often of much practical aid in identification when only fragments of the plant or animal are available for study.

(4) The part of the organism utilized by the natives, *i.e.*, root, stem, leaf, flower, seed, etc.

(5) The purpose for which the plant or animal or part thereof is utilized.

(6) The exact manner in which the organism or the part used is prepared.

(7) The season in which the plant or animal is collected by the natives. This contributes toward working out the calendric sequence or annual cycle with respect to economic life.

(8) Whether or not the organism is native to the area inhabited by the people under consideration.

(9) The antiquity of utilization. It is of the utmost importance to ascertain whether or not such employment of a specific organism is aboriginal.

(10) The actual value by modern standards of the plant or animal employed. This applies particularly to the study of plants used medicinally and involves an inquiry into the actual therapeutic value of the plant. Frequently such information is not available in our own materia medica and would call for pharmaceutical research, which of course lies outside the purview of the ethnobiologist.

(11) What species of plants and animals in the tribe's environment are not utilized. For example, as indicated above, some tribes ignore major, readily available food sources. Such facts are significant, call for explanation and may illuminate certain cultural relations.

(12) Importance of a specific plant or animal in the tribe's economy. Frequently, published papers merely state that a people utilized a certain organism, and such information is practically worthless. It is of the utmost importance to weigh the value of specific plants and animals in exact terms, if possible, otherwise relatively. The ideal would be to ascertain, for example, whether a given wild or cultivated plant constitutes 5, 30 or 70 per cent. of a tribe's total food supply.

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REVIEWS AND COMMENTS

EDITED BY CARL L. HUBBS

IN this section reviews and notices are given of current publications on general biology and of specialized works which have an important bearing in this general field. Emphasis is given to books and major articles which fall within the special scope of THE AMERICAN NATURALIST, in that they deal with the factors of organic evolution.

REVIEWS AND COMMENTS are meant to include also such general discussions, reports, news items and announcements as may be of wide interest to students of evolution. Except as otherwise indicated, all items are prepared by the Section Editor, Dr. Carl L. Hubbs, University of Michigan, Ann Arbor, Michigan. All opinions are those of the reviewer.

Finger Prints, Palms and Soles. By HAROLD CUMMINS AND CHARLES MIDLO. Philadelphia: The Blakiston Co., 1943: i–xi, 1–309, frontisp., figs. 1–149. $4.00.

THE value of finger prints in individual identification is widely recognized. Their biological significance, as well as that of palms and soles, has been just as widely neglected, even by the majority of biologists. Nevertheless, in studying these characters, some few scientists, since the time of Francis Galton, have uncovered fascinating and surprisingly large amounts of information bearing on problems of embryology, physical anthropology, genetics and evolution. The general lack of interest in and knowledge of dermatoglyphics has likely, in no small measure, been due to the lack of any book or journal comprehensively dealing with the various aspects of the subject. This lack has recently been eliminated in an admirable manner by the publication of Cummins and Mildo's new book, "Finger Prints, Palms and Soles."

The book consists of three parts: I, Orientation; II, Methodology and Description; and III, Biology. Parts I and II acquaint one with the history and techniques of dermatoglyphics. Part III gives a very complete and comprehensive discussion of the various aspects of dermatoglyphics, of interest to biologists. Practical applications, as in twin diagnosis and questioned paternity, are carefully considered. The chapters on comparative dermatoglyphics, embryology, symmetry, inheritance, racial

variation and constitution furnish an abundance of material which should be of interest to the student of evolution. The following paragraphs give a brief résumé of some of the authors' conclusions:

The pattern areas are elevated regions on finger-tips, palms and soles. These are homologues of the primitive volar or walking pads of other mammals. The epidermal ridges are derived from scales, each of which was associated with a sweat gland and a hair. During the evolution of the epidermal ridges the hairs disappeared, and the scales aggregated into ridges. Some degree of ridge formation is manifest on the volar pads of all primates. The degree of pattern development is positively correlated with the degree of volar pad elevation. In both palms and finger-tips the evolutionary trend has been from high pattern intensities to patternless configurations. Of pattern types, elliptical whorls are the most primitive, followed by circular whorls. Arches are the most specialized. At the close of the second month of embryological development the volar pads of the palm and fingers appear as localized bulges, and during the ensuing four weeks become more rounded and individualized. At about the thirteenth week these pads begin to recede and become more indefinite. Pads of the sole develop and recede about two weeks later than those of the hand. Epidermal ridges of the fingers are differentiated during the third and fourth fetal months, and elevated on the skin surface at about eighteen weeks. Finger ridges appear first and sole ridges last. If a pad does not completely recede before ridge information, a pattern will be formed in the area. If it does recede before ridge formation, the area will likely be patternless. The three great apes and man present specializations which follow different directions. The evidence indicates that man stemmed from an ancestral stock more primitive than any recent ape, having dermatoglyphic traits more closely allied to those of monkeys.

Genetic factors are responsible for much of the varia-

tion in dermatoglyphics, as evidenced by the great intra-pair similarities of identical twins, and lesser similarities between less closely related individuals. The genetics of the pattern intensity variations in finger-tips is the most thoroughly understood, although the inheritance of certain variations in finger pattern shape and the occurrence of thenar-first interdigital patterns appear to be fairly simple. Interracial comparisons manifest marked differences in gene frequencies. Accepting whorl-loop-arch as representing successive departures from the primitive, finger prints of the yellow-brown human races are the most primitive. The most specialized are certain components of the black, and among whites, the Nordics. Geographically in Africa, Asia and America there is a tendency towards reduction of pattern intensity from north to south, whereas in Europe the line of pattern reduction is from south to north.

Dermatoglyphics are differentiated by the end of four months of fetal development and undergo no further changes, except in total size, during the life of the individual. Consequently, any associations between dermatoglyphics and constitutional types are indicative of differences existing during early fetal development. Several such associations are known. Patterns show consistent differences in their occurrence on right and on left hands. Populations of left-handers show a reduction of bimanual asymmetry, as contrasted with populations of right-handers. Males show greater variability than do females. The dermatoglyphics of Mongolian idiots deviate markedly from those of normal individuals. Finger-print trends also appear to be aberrant among victims of neurofibramatosis, schizophrenia and epilepsy.

The book is amply illustrated. A bibliography at the end includes 361 references. The appearance of "Finger Prints, Palms and Soles" should do much to stimulate interest in this field, so rich in biological significance, which in the past has often been overlooked or neglected.

D. C. RIFE

Taxonomy and Phylogeny. By W. B. TURRILL. Bot. Rev., 8, 1942: 247–270; 473–532; 655–707.[1]

WHEN a long-felt need has been successfully met the achievement certainly deserves recognition. In the opinion of the present writer, this review of Turrill's does meet a real need on the part of younger students who desire orientation in the maze of plant classification as well as older ones, most of whom have concentrated more or less narrowly on the systematics of certain groups of organisms. Although the title is general, the author is a botanist and "conclusions or opinions of zoologists are quoted only when they supplement or contrast with those of botanists." The review consists of three parts.

Part I, after an illuminating discussion of the derivation and meaning of terms, especially taxonomy and phylogeny, briefly reviews the historical development of plant classification, from pre-Linnaean to post-Darwinian systems. Part II reviews (1) taxonomic and phylogenetic concepts and criteria; (2) data used in classification and phylogenetic studies. Part III presents (1) a brief discussion of each of the major groups of both cryptogams and phanerogams; (2) general considerations and conclusion.

It is impossible in a brief note to give an adequate notion of the content of this paper. No serious student of systematics can afford to ignore it and, for all younger students in this field, it may well serve as an introductory text. The amount of literature covered is extensive and truly representative in each of the several fields. The organization is logical, the style is clear and readable, and the author's interpolations of his own ideas and opinions are critical and constrained. There is much of interest to any biologist who is at all concerned with evolution.

Certain parts that particularly interested the present writer are: Darwin in the history of plant classification,

[1] This contribution inadvertently failed to receive current review in our columns, but is judged to be of such great significance as to warrant this belated notice.—C. L. H.

pp. 264–266; the species concept, pp. 479–482; principles of phylogeny, pp. 488–495; data used in classification and phylogeny, pp. 496–532; and discussion of "logical as opposed to phylogenetic classification," pp. 676–684.

The last-mentioned topic is in itself somewhat unfortunate since it implies that no phylogenetic classification can be logical, which is not necessarily true. But the discussion which follows is a sane, well-balanced evaluation of the general problem of maintaining a *practial* system of nomenclature for organisms and at the same time giving due regard to the generally accepted principle that these organisms have evolved and are now evolving. Quoting Turrill's concluding paragraph:

> The task of the systematist is twofold: to prepare as many special classifications as are needed for special biological investigations, or to make available materials for others to construct such special classifications, and to make a general classification which shall express as far as possible in rational order all that is known concerning plants and animals. This last is an ideal which, even if never attained, is one which may well make the systematist proud in the magnitude of his task. It is an ideal greater than the phylogenetic ideal which is included in it and one which in the process of attempted attainment must make taxonomy what it should be, the focal point of biology.

E. B. Babcock

Notices of New Books

A Cytological Study of *Crepis fuliginosa, C. neglecta,* and Their F_1 Hybrid, and Its Bearing on the Mechanism of Phylogenetic Reduction in Chromosome Number. By H. A. Tobgy. Jour. Genetics, 45, 1943: 67–111, pls. 1–3, figs. 1–74.—Dysploid series of chromosome numbers, such as 6, 5, 4, 3, among closely related species have been assumed to arise through translocations between non-homologous chromosomes followed by the loss (or addition) of a chromosome pair (Navashin's "dislocation hypothesis"). Until now the only evidence in support of this hypothesis is Dubinin's experimental production of a 3-paired form of *Drosophila melanogaster* (cf. Biol. Zhur., 3, 1934: 719–736). The first supporting evidence from plants was found by Dr. Hassan A. Tobgy of the Egyptian University, Cairo. From a careful analysis of meiotic phenomena in F_1 hybrids between

two wild species, *C. fuliginosa* ($n=3$) and *C. neglecta* ($n=4$), Tobgy has been able to show that two chromosomes, designated B and C, in the 4-paired species are represented by just one chromosome (B) in the 3-paired species. The reduction in number is assumed to have come about through unequal reciprocal translocation between the B and C chromosomes of *C. neglecta,* in such a way that the C centromere was left with only genetically inactive chromatin attached to it, and through the eventual loss of this C centromere. Additional evidence supporting this assumption is presented. Coming from natural species in a genus where progressive reduction in chromosome number is the general rule, this achievement reveals the most probable cause of dysploid series in general. The Division of Genetics of the University of California, where the research was done, will be in position to supply reprints to those who care to apply for them.—E. B. BABCOCK.

An Analysis of Mink Predation upon Muskrats in North-Central United States. By PAUL L. ERRINGTON. Res. Bull. Agri. Exp. Sta. Iowa State Coll., 320, 1943: 797–924, figs. 1–16.—Although undertaken primarily as an investigation in the field of wildlife management, this research like others by the same author penetrates deeply into the philosophy of predation and bears directly on problems of population analysis and indirectly on problems of speciation. "It was found that strangeness of environment, intraspecific intolerance, and drought were especially important in predisposing muskrats . . . to predation . . . ; and that kinds and numbers of wild predators doing the preying, with a few apparent exceptions, had little bearing upon the net mortality. . . . For reasons of intercompensatory (automatically adjusting) trends existing in reproductive as well as loss rates of the muskrats, severity of predation . . . may leave highly misleading impressions as to population effect. Even when locally nearly annihilative, predation rarely showed evidence of functioning as a true population depressant, insofar as it usually only took the place of some other mortality factor." Opponents of the theory of natural selection, like McAtee, may find in such studies support for their position. If loss to a predator merely releases the need for intraspecific population control, if the prey becomes invulnerable to predation but continues to exist under severe predation loss, if it is inconsequential which species of

predator is doing the preying, and if removal of predators will have little effect on the populations of the prey, then what significance can be attached to any protective device, such as concealing coloration or special habits, which seems to have been evolved to prevent the destruction of the species by any one predator, or by predators in general? Here's something to think about. Obviously the life or death of a species depends on a highly complex adjustment to a multitude of potentially destructive factors. Perhaps one of the leading adaptations has been the perfection of the "intercompensatory trends."

Northern Fishes With Special Reference to the Upper Mississippi Valley. By SAMUEL EDDY AND THADDEUS SURBER. Minneapolis: University of Minnesota Press, 1943: i–xi, 1–252, frontisp., figs. 1–57 (4 col.), diags. 1–3. $4.00.—"Northern Fishes" will help ichthyology to catch up with other phases of American vertebrate zoology. Though it was written as a Fishes of Minnesota, it will be needed by every student of the fishes of any part of eastern North America, and warrants a good sale among general naturalists and among sportsmen. The introductory chapters are well written. The nomenclature is up-to-date and the general statements on characters, habits and on distribution are valuable and interesting. The authors hit a good batting average of accuracy, though minor misstatements and omissions will be occasionally apparent to the specialist. The greatest scientific contributions perhaps lie in the analyses of the distribution of the various species in Minnesota. Of most general biological interest is the account of the silver pike, a true-breeding mutant of the northern pike which has appeared in recent years in a number of Minnesota lakes. This peculiar fish is beautifully illustrated in color, along with a muskallunge. The three other colored plates are hardly up to modern standards. The numerous photographs, however, are singularly successful in portraying specific characters.

The Green Earth An Invitation to Botany. By HAROLD WILLIAM RICKETT. Lancaster, Pa.: Jaques Cattell Press, 1943: 1–350, many figs. $3.50.—One of the Humanizing Science Books of The Jaques Cattell Press, "The Green Earth" is offered as "a layman's guide to botany." For this purpose it is beautifully written, with skillful choice of plain words, with lucid descrip-

two wild species, *C. fuliginosa* ($n=3$) and *C. neglecta* ($n=4$), Tobgy has been able to show that two chromosomes, designated B and C, in the 4-paired species are represented by just one chromosome (B) in the 3-paired species. The reduction in number is assumed to have come about through unequal reciprocal translocation between the B and C chromosomes of *C. neglecta,* in such a way that the C centromere was left with only genetically inactive chromatin attached to it, and through the eventual loss of this C centromere. Additional evidence supporting this assumption is presented. Coming from natural species in a genus where progressive reduction in chromosome number is the general rule, this achievement reveals the most probable cause of dysploid series in general. The Division of Genetics of the University of California, where the research was done, will be in position to supply reprints to those who care to apply for them.—E. B. BABCOCK.

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tions and with impressive figures of speech. It would, for example, be difficult to find a better-worded statement on the search for the basic principle of life than this: "Biologists and philosophers, each in turn misleading the other, have trampled the ground into a morass upon which it profits not to venture." The author's competence as an artist does not end with his word pictures, for he has very attractively illustrated his book with pen and pencil sketches of plants and their parts.

When early in the reading I encountered the false statement that the smallest fish live on the plants, I began to look for further errors, but soon ended the search with the conviction that the text is marked by accuracy as well as beauty. Nontechnically but reliably the competent author writes of photosynthesis, of leaf anatomy, of the architecture of plants, and of their growth; inserts the philosophical chapter, "Of the Purposes of Plants," from which the quotation is taken; then describes flowers and fruits, plant genetics, and plant nomenclature; finally races through a discussion "Of Fern and Moss" and of seed plants, to finish with "The End of Things" and a thoughtful Epilogue.

Manual of Comparative Anatomy. By OSMOND P. BRELAND. New York and London: McGraw-Hill Book Co., 1943: i–ix, 1–250. $2.00.—Text books and laboratory manuals in comparative anatomy keep coming from the press in a seemingly endless flow. This is a good manual—like many of the others. The very explicit instructions, especially for the drawings, will save the student from any need for mental effort or originality in planning his work, and will lighten the instructor's load. The treatment is purposefully conventional in vertebrate classification and is similarly conservative in anatomical and systematic nomenclature. The dogfish is stressed, as a simple example of fundamental vertebrate organization; amphioxus, lamprey, perch, *Necturus* and turtle are treated in less detail. Lastly the cat is accorded more attention, as an example of mammalian anatomy. Occasional paragraphs help justify calling this a manual of *comparative* anatomy. There are no illustrations. The typography is beautifully clear and the reduced over-all size of the page, though pardoned as a required war economy, strikes me as an advantage.

Vertebrate Photoreceptors. By SAMUEL R. DETWILER. New York: Macmillan Co., 1943: i–x, 1–184, figs. 1–110. $4.00.—"The eyes have it." Excellent general treatises on the vertebrate

eye and on vision continue to appear. Among the more recent (1941–1943) items, four may be singled out for mention. The 1941 contribution, "Vision A Study of Its Basis," by S. Howard Bartley (New York: D. Van Nostrand Co., 1941: i–xv, 1–350, figs. 1–77. $3.50), is a well-documented handbook emphasizing the marked advances of the past decade in the physiology of vision. In 1942 two outstanding volumes appeared: "Visual Mechanisms (Biological Symposia, Vol. VII, noticed in the September-October, 1942, number of The American Naturalist) and "The Vertebrate Eye and Its Adaptive Radiation," by Gordon L. Walls (reviewed in our columns for January–February, 1943).

The most recent of these books is Detwiler's Vertebrate Photoreceptors. It is issued in the Macmillan series of Experimental Biology Monographs, but gives at least as much attention to comparative anatomy and histology of the eye as to physiological experiments. Bartley's book on Vision is not referred to, and Wall's compendium is barely mentioned. Technical contributions, however, are extensively cited. Detwiler's expressed aim has been "to present a general account of the visual cells of vertebrates, with particular emphasis upon their development, structure, distribution, and certain aspects of their physiology." He pays particular attention to the duplicity theory, which has been sustained, he thinks, by quantitative researches on the major functions of vision: dark adaptation, intensity discrimination, visual acuity and flicker responses. He summarizes researches on these functions, stressing in particular Crozier's recent work on flicker responses. He also emphasizes the researches by Wald on the visual pigments and their relation to the A vitamins.

Microscopic Technique in Biology and Medicine. By E. V. Cowdry. Baltimore: Williams and Wilkins Co., 1943: i–iv, 1–206. $4.00.—Concentration and organization are key features of this handbook. Beyond the brief introduction, dealing with choice of methods, organization of laboratory and standardization of stains, the subject matter is arranged alphabetically. Here one will find under the names of stains, fixing agents and other chemicals, brief statements as to their use; under anatomical and cytological terms, suggestions for effective treatment; under animal names, techniques particularly suited to the given group; under authors' names, special methods proposed by these tech-

nicians. Throughout, the statements are marked by clarity and brevity, and are abundantly documented by references to the source literature. Cross references are frequent. Only a great, productive investigator would have conceived such an arrangement, or would have done the job so well. Outstanding is the stress on new methods, which are based on recent advances in physics and chemistry and which are opening new avenues of research and interpretation.

Frontiers in Cytochemistry The Physical and Chemical Organization of the Cytoplasm. Edited by NORMAND L. HOERR. Biological Symposia, Vol. X. Lancaster, Pa.: Jaques Cattell Press, 1943: i–vii, 1–334, frontisp., pl. 1, 98 figs. $3.50.—What finer monument could a scientist want, than a printed symposium like this one, dedicated to him out of deep respect for his pioneering and his leadership in a fundamental field of research? Particularly fine is the indication that the recipient of this honor, Dr. R. R. Bensley, has made some of his most notable contributions during the decade following his retirement to an emeritus professorship. May his example be emulated!

This symposium presents the results of the epochal advances made during the past few years in the knowledge of the physics and chemistry of cytoplasm—advances due largely to Bensley's active participation and intellectual leadership in the research, to his constant search for new analytical methods and to his insistence on maintaining the cellular elements in as little modified and as little disorganized a condition as is consistent with their separation and analysis. Outstanding elements in this advance include the mass separation of mitochondria, the recognition of such bodies as the submicroscopic lipoprotein complex and particulate glycogen, and the actual discovery of the theoretically assumed micellar components.

An Introduction to Biophysics. By OTTO STUHLMAN, JR. New York: John Wiley & Sons, 1943: i–vii, 1–375, 154 figs. $4.00.—In these days of integration of sciences, special education in the boundary fields becomes increasingly important. This text provides for training in biophysics. It is written by a physicist, but the text as well as the bibliographies which accompany each chapter are rich in references to biological work. The 8 chapters deal with biophysically active x-rays, applied radioactivity, bio-

physical characteristics of the eye, emission and absorption of biophysically active light, the structure and properties of surfaces and membranes, the biophysical problem of nerve conduction, auditory biophysics, and lastly, with the compound and electron microscopes.

Why the Universe? Or, Cosmopoietic Space. By PERCY A. CAMPBELL. San Francisco: George Fields, 1943: 1–189. $2.50.—Often when tempted to write disparagingly of a man's printed work I find myself restrained by the attitude of an older investigator when commenting on an author whose treatise might well have been ridiculed. "At least," he said, "his mind has not been stagnating." Certainly no one could accuse the author of "Why the Universe?" of mental stagnation. He starts with the assumption that space itself is the unitary principle of the universe, and believes that space can and must "be given also the all inclusive cosmopoietic role, the role of cosmos maker." Physics and biology, evolution and anthropology, conservation and religion: he discusses them all. And he pictures life as "self-aggrandizing bioprecipitation."

Handbook of Salamanders. By SHERMAN C. BISHOP. Ithaca, N. Y.: Comstock Publishing Co., 1943: i–xiv, 1–555, colored frontispiece, figs. 1–144, maps 1–56. $5.00.—This volume, the best of the "Handbooks of American Natural History," is as momentous to herpetology as was Cope's "Batrachia" (1882) and Blanchard's "Key to the Snakes" (1924). An admirable introduction of some twenty pages, followed by an account of the species and subspecies, is buttressed by the index and an excellent 67-page bibliography. One hundred twenty-six species and subspecies are recognized, including 100 of the 102 recognized in the Stejneger and Barbour "Checklist" of 1939.

That the author knows and is in sympathy with his subject and that he has not been commissioned to present it is refreshingly apparent throughout. Although the manuscript was in preparation for many years there is nothing labored about the finished product.

For the systematic accounts, the life-histories, the keys, the many and excellent illustrations the reviewer has only the highest praise. The outstanding faults of the fine handbook all concern the distribution maps, which are ill-chosen in regard to size, com-

position and symbols. Maps showing actual locality records would have been much more serviceable and thought-provoking than the general distribution maps, often misleading, employed in this volume. Whether a map portrays a restricted distribution of a single form or the widespread distribution of several forms the type and size of the maps are the same. Indications of latitude and longitude are omitted. The choice of maps was apparently haphazard, for of the 56 maps, 35 show the major rivers of the areas portrayed while these features for no apparent reason are omitted on 21.

These minor faults detract but little from the immense value of the book. Certainly every herpetologist must have a copy and every naturalist should.—NORMAN HARTWEG.

GENETICS SOCIETY OF AMERICA

THE Genetics Society of America announces that the following officers have been elected for 1944: President, Professor A. H. STURTEVANT, California Institute of Technology, Pasadena; Vice-President, Dr. B. P. KAUFMANN, Carnegie Institution, Cold Spring Harbor, New York; Secretary-Treasurer (for a term of three years), Professor LAURENCE H. SNYDER, Ohio State University, Columbus. These three officers, together with the two most recent past presidents of the society, Professor M. M. Rhoades, Columbia University, and Professor E. W. LINDSTROM, Iowa State College, constitute the Executive Committee for 1944.

A recent survey conducted with a view to revising the directory of the society shows that about ten per cent. of the members are now serving in the armed forces, and that a considerably larger number are engaged in scientific work directly related to the war effort. No formal meetings of the society have been held during the past two years, although other activities, including publication of the "Records," continue. The current issue (No. 12) of the "Records" contains, in addition to the directory of members and reports on the activities of the society during 1943, a series of short papers dealing with recent genetic studies.

B. P. KAUFMANN

SHORTER ARTICLES AND DISCUSSION

THE GENETIC BASIS OF DIFFERENCES BETWEEN TWO SPECIES OF DROSOPHILA

THE problem, whether hereditary differences between different, related species are due exclusively to differences in their chromosomes or whether non-chromosomal, cytoplasmatic elements are involved also, has found various answers. Among plants, convincing evidence has been presented for some cases in support of the view that only Mendelian inheritance by genes is responsible for species differences, while in others extra-chromosomal elements clearly have a share in this determination. In animals few cases have been amenable to treatment, this being largely due to the interference of hybrid sterility, which places a bar before a genetic analysis.

The two closely related species *Drosophila virilis* Sturtevant and *D. americana* Spencer offer suitable material for a test of this nature. These members of the "species complex *Drosophila virilis*" were originally considered subspecies and are so listed by Sturtevant (1942) while Patterson (1943) regards them as distinct species. We shall follow the latter author's designation. While very similar to each other the two forms are morphologically distinct and reproductively strongly isolated (Spencer, 1940, Patterson, Stone and Griffin, 1940, 1942, Stalker, 1942a). Hybrids, however, can be obtained and are partially fertile. This makes it possible, by repeated backcrosses of the hybrids to one of the original species, to obtain individuals in which all chromosomes are derived from this latter species. If the cross is so arranged as to have the cytoplasm of these individuals descended from the other species of the original cross the following alternatives can be decided upon:

(a) The specific differences between *virilis* and *americana* are carried exclusively by the chromosomes. In this case the backcross individuals should completely resemble that species whose chromosomes they contain.

(b) The specific differences are wholly, or partly, due to self-reproducing cytoplasmatic elements. In this case the backcross individuals should retain characteristics of that species, none of whose chromosomes, but whose cytoplasm they contain.

Obviously, the experiment can be arranged in two ways, namely

to make either *americana* the cytoplasmatic and *virilis* the chromosomal donor or reciprocally. Only the first type of experiment was carried out, in three parallel sets. In two of them *americana* females in mass cultures, were mated to *virilis* males (of the sepia yellow scute stock), the F_1 females, again in mass matings, crossed back to *virilis* males, and single females of the next generation mated again to single *virilis* males. In the third line single pair matings were used from the very beginning. In all three lines further backcrosses of the type described, in pair matings, were repeated over a period of about two years. At the end of this time three separate lines of "virilized" flies, derived from three original *americana* females, were available. These females came from stocks descended (1) from a single fertilized female collected by H. D. Stalker at Defiance, Ohio, and (2) from another such female collected at Overton, Ohio. The three strains of backcrossed hybrids will be referred to as Ad (from Defiance), Aoa and Aob (each from a separate female of the Overton stock). In order to compare the chromosomally *virilis*-type hybrids with pure *virilis* the experiment was conducted so as to have available a *virilis* stock chromosomally highly isogenic with the hybrids. It was intended at first to establish three separate *virilis* strains each isogenic with a different one of the hybrid strains. Accordingly, the single *virilis* male used in each backcross was also used in a separate culture bottle, in a mating to a single pure virgin *virilis* sister, and the parents of the next generation were taken from among the offspring of the *virilis* male with (1) its hybrid and (2) its *virilis* mate.[1] After from three to seven such separate hybrid female × *virilis* male × *virilis* sister crosses it was decided to make all three hybrid strains isogenic among themselves and with a single *virilis* strain. This goal was approached by the following sequence of single pair matings:

generation "n"	hybrid Ad ♀ × vir ♂ × vir sister
	hybrids Aoa ♀ and Aob ♀ × brothers of above vir ♂
generation "n + 1"	hybrid Aoa ♀ × vir ♂ (F_1 of generation n) × vir sister
	hybrids Aob ♀ and Ad ♀ × brothers of preceding vir ♂
generation "n + 2"	hybrid Aob ♀ × vir ♂ (F_1 of generation n + 1) × vir sister
	hybrids Ad ♀ and Aoa ♀ × brothers of preceding vir ♂

with generation "n + 3" repeating the type of cross of generation "n," "n + 4" repeating "n + 1" etc. Since this scheme was

[1] We are indebted to Mr. Lloyd Birmingham, who carried out the initial crosses.

practised for at least 23 generations a high degree of similarity in the chromosomally determined genetic makeup of the three hybrid lines and the *virilis* line must have been obtained. A cytological investigation of the salivary glands in the hybrid lines, while obviously unable to verify isogeneity in its strictest sense, nevertheless confirmed the expectation of at least no appreciable segment of any *americana* chromosome being present. Segmental heterogeneity would have been obvious from the appearance of incomplete pairing of sections of the chromosomes or of inversion loops; however, pairing was normal in the few cases of each line studied.

Comparisons between the three hybrid and the *virilis* lines involved both morphological and physiological characters used in the specific diagnosis of the two species. With the exception of two of these characters, cross-fertility and position of flies, these comparisons were carried out by the one of us (W. P. S.) who first described *americana* and pointed out its relation to *virilis* (Spencer 1938, 1940; see also Stalker, 1942a). A morphological comparison relating to (1) width of cheek, (2) eye pile, (3) eye size and (4) shape of spermathecae yielded no recognizable differences between any of the three hybrid lines and *virilis*. Another character, (5), width of carina, which, due to overlap in the two species, is a less reliable differentiator, also appeared alike in all four stocks. These comparisons were made after 33 generations of single pair backcrosses in the lines Ad and Aoa, and after 29 such generations in Aob (plus the initial two generations of mass matings in Ad and Aob). Since three additional morphological characters, namely (6) body color, (7) eye color and (8) cloudiness along veins of wing, could not be studied in the isogenic lines, owing to the presence of the mutants sepia and yellow, individuals from all four lines were mated to wild type *virilis* from a stock culture and the F_1 flies compared among each other (this outcross was undertaken one backcross generation later than the time of the first comparisons). Again there was every indication that the three hybrid lines were identical to *virilis* and different from *americana*. A striking physiological differentiation between the two species, (9) speed of etherization and recovery from ether, also appeared alike in the hybrids and in *virilis*. The same was true for (10), position of undisturbed adults in culture bottles: Unlike *americana*, which form closely grouped aggregates, the hybrid strains showed the adults in the isolated

positions characteristic for *virilis*. All ten specific character differences mentioned can thus be said to be due to chromosomal differences between the two species only. The detailed basis of these differences is unknown, but an eleventh character, coloration of pupae, has been shown by Stalker (1942b), to depend on differentiating genes located in chromosomes (2 . . . 3) and 5 of *americana*.

Since the low fertility of the cross between *americana* and *virilis* furnishes an important argument in the assignment of specific rank to the two forms, a quantitative study was made of the ability to produce offspring in crosses between the hybrid lines and the *virilis* line. Pair matings of 100 (in one case 82) females

TABLE 1

PERCENTAGE OF FERTILE CULTURES IN PAIR MATINGS OF INDIVIDUALS WITHIN HYBRID LINES, WITHIN THE VIRILIS LINE AND BETWEEN HYBRID FEMALES AND VIRILIS MALES. ALL DETERMINATIONS BASED ON 100 CULTURES EXCEPT IN THE CROSS AOB ♀ × VIR ♂ WHERE 82 CULTURES WERE AVAILABLE

P–♀	Percent of fertile cultures P–♂	
	from hybrid line	from virilis line
Ad	90	93
Aoa	92	94
Aob (exp. 1)	78	78
virilis (exp. 1)	..	91
Aob (exp. 2)	..	87
virilis (exp. 2)	..	83

Matings reported in rows 1–4 were conducted after 30 generations of single pair matings in lines Ad, Aoa and virilis, and after 26 single pair matings in Aob. Matings reported in rows 5 and 6 occurred three generations later.

from the three hybrid lines with *virilis* males were arranged for and the percentages of fertile cultures compared with those from 100 control pair matings of females and males within each of the hybrid lines as well as with the fertility frequency in 100 *virilis* pair matings (Table 1). The left and right columns of the first three lines in the table show that no significant difference between self-fertility of any line and its cross fertility with *virilis* existed. The percentages of fertility for matings involving hybrid Ad and Aoa females are only slightly below those determined for frequency of insemination of pure Ad and Ao females by males of the same strain (96.8 and 97.5 per cent., respectively, Stalker 1942a), and very much higher than the frequencies of interspecific inseminations in these strains as observed by the same author (10.7 and 12.3 per cent.). A further comparison of the

fertility of crosses involving individuals from hybrid lines with that within the *virilis* line gave no significant differences in the cases of hybrid Ad and Aoa females (90–94 per cent. as compared with 91 per cent. Stalker found 97.7 per cent. of insemination within *virilis* matings). However, both matings involving Aob (table 1, line 3) yielded significantly lower fertility than any of the others. Most of the sterile pairs of the matings Aob × Aob belonged to one or the other of two groups of sibs while the fertility in the remaining pairs, belonging to six groups of sibs was over 90 per cent. as in the other matings. Similarly, fourteen females of the eighteen sterile pairs of the cross Aob × vir belonged to one single group of 25 sibs. It was also found that six of the ten sterile females in the mating Ad × Ad were derived from a single sibship from which seven pairs were set up. These facts show that the sterility found can not be regarded as an intrinsic character of the lines tested but was either due to segregating residual genetic heterogeneity or perhaps rather to unknown environmental circumstances.

In order to test the assumption that the lower fertility in line Aob was incidental, the cross Aob × vir was repeated and a new set of vir × vir matings was arranged as control (Table 1, lines 5 and 6). This time no significant difference in percentage of fertility was observed. The fertility of the outcross approached that of the majority of the earlier lines and surpassed that of the new *virilis* matings. It may be concluded that the frequency of fertile matings between females and males of hybrid origin does not differ from the frequency of fertility in the cross of such females with pure *virilis* males. Thus, all morphological and physiological tests yield the result that the specific differences between *Drosophila virilis* and *D. americana* do not depend on any self-reproducing extra chromosomal elements but on the genetic constitution of the chromosomes of the two species.

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RECOMBINATION AND INTERFERENCE NEAR THE LEFT END OF THE SEX CHROMOSOME OF HABROBRACON[1]

STARTING at the left end of the sex chromosome of *Habrobracon juglandis* (Ashmead), the genes are arranged in the order: speckled (*Sk*, an eye color factor), reduced (*r*, a wing size and vein factor) and glass (*gl*, a structural eye factor). The present experiment was done in order to check recombination and interference in this region, and to compensate for any viability disturbance or position effects by introducing the genes in all possible combinations. In Habrobracon, diploid females produce haploid sons parthenogenetically. Hence, in linkage work, typical testcross ratios are obtained by crossing a female homozygous for each possible gene combination to a complementary male, and breeding the F_1 heterozygous females from this cross as virgins. Throughout this work, all stocks carried the mutant, white eye, as a differentiator for speckled. White is not located on the sex chromosome. The number of possible combinations of *Sk*, *r* and *gl* is limited because *gl* is female sterile and had always to be introduced from the male, and also because *gl is* epistatic to *Sk*.

Table I[2] gives the results of the four possible crosses. In the genotype of the F_1 mother (see col. 2) the gene complement of the parental female is above the line, that of the parental male, below. The non-glass genotypes of the F_2 males (col. 4) were distinguishable, but the glass genotypes (col. 7) could be separated only on the wing character, since speckled does not show in a glass eye. Among the non-glass, the ratios of recombination between *Sk* and *r* (region 1) and between *r* and *gl* (region 2) were computed and appear in column 6 following the singles in the

[1] The author wishes to acknowledge the assistance and encouragement of Dr. P. W. Whiting and Dr. G. M. McKinley.

[2] Part of these data were included in a University of Pittsburgh dissertation (1942).

given region. Also in this column are the coincidence values following the doubles. Among the glass sons, one of the combined classes contains region 2 recombinations and double recombina-

TABLE I

Exp.	F_1 mother	Recomb. Regions	Non-glass F_2 males		Ratios of Recomb. & Coincidence	Glass F_2 males		Ratios of Recomb.
1	$\frac{Sk}{r\ gl}$	–	Sk	1,977		Sk r gl and r gl	2,048	
		1	+	284	0.106			
		2	r	448	0.167	Sk gl and gl	453	0.179
		1, 2	Sk r	4	0.084			
		Totals		**2,713**			**2,501**	
2	$\frac{Sk\ r}{gl}$	–	Sk r	2,620		Sk gl and gl	3,101	
		1	r	467	0.135			
		2	+	502	0.140	Sk r gl and r gl	494	0.132
		1, 2	Sk	20	0.284			
		Totals		**3,609**			**3,595**	
3	$\overline{Sk\ r\ gl}$	–	+	1,381		Sk r gl and r gl	1,469	
		1	Sk	229	0.127			
		2	Sk r	227	0.125	Sk gl and gl	190	0.113
		1, 2	r	2	0.067			
		Totals		**1,839**			**1,659**	
4a	$\frac{r}{Sk\ gl}$	–	r	463		Sk gl and gl	330	
		1	Sk r	44	0.093			
		2	Sk	35	0.077	Sk r gl and r gl	42	0.088
		1, 2	+	7	1.794			
		Totals		**549**			**372**	
4b	$\frac{r}{Sk\ gl}$	–	r	1,764		Sk gl and gl	1,490	
		1	Sk r	263	0.126			
		2	Sk	133	0.067	Sk r gl and r gl	183	0.109
		1, 2	+	12	0.654			
		Totals		**2,172**			**1,673**	
	Total count	–		8,205			8,438	
		1		1,287	0.122			
		2		1,345	0.128		1,362	0.139
		1, 2		45	0.264			
		Totals		**10,882**			**9,800**	

tions, and hence could be used to determine the ratio of recombination between *r* and *gl*. However no other direct determinations could be made from the glass data.

The results for crosses 1, 2 and 3 are statistically consistent. In each cross, the non-glass are more numerous than the glass, probably because of better viability and hatchability. The recombination ratios for region 2 based on the non-glass data are reasonably close to those based on the glass data. It is interesting to note that the amount of interference varies markedly with different genic combinations.

The results of cross 4a differ appreciably from those of the previous crosses. The recombination ratios between *Sk* and *r* and between *r* and *gl* are significantly lower than the values obtained in crosses 1, 2 and 3. Moreover, there is a disproportionate number of double recombinations, giving a coincidence value indicative of negative interference; whereas the other crosses showed positive interference. Possible explanations of these differences are: (1) the count in cross 4a is low and hence might not be a representative sample; (2) the double recombination class is type and might therefore be more viable. Several unusual reduced wasps appeared in this cross. In pure stock, reduced shortens the wing and removes that portion of the median vein (Ross system) that runs toward the stigma. Wasps were observed with short wings and some irregularity in the median vein but with the entire vein present.

Cross 4b was run as a check. A sufficiently large number of wasps was counted in order to assure a representative sample. The recombination ratio between *Sk* and *r* agrees with the values obtained in the other crosses, but one of those between *r* and *gl* is significantly different, the one based on the non-glass data; the recombination ratio based on the glass data is consistent with the results of the other crosses. This difference within the cross arises from a deficiency of *Sk*. In this cross, as in 4a, the coincidence value is significantly higher than in crosses 1, 2, and 3. There were 48 wasps with unusual reduced wings. Since this odd expression of the factor, reduced, was not observed in any of the other crosses, it might be ascribed to a position effect of the genes.

Possibly the most representative values for recombination and coincidence in the *Sk, r, gl,* region are obtained by totaling the data. Such a grouping compensates for proportionate disturbances due to viability differences or position effects of the gene combinations involved.

SUMMARY

Sk, *r* and *gl*, three genes marking the left end of the sex chromosome of Habrobracon, are introduced in the four possible combinations. The ratio of recombination between *Sk* and *r* is 0.122; that between *r* and *gl* 0.128. Coincidence is 0.264.

Coincidence varies markedly as the genes are introduced in different combinations.

The recombination ratios obtained in 4a and 4b differ significantly from those obtained in the other three crosses.

Several unusual reduced wasps appeared in crosses 4a and 4b but in no other crosses.

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THE POLYTYPIC SPECIES IN NORTH AMERICAN AMPHIBIANS AND REPTILES

IN Dr. Mayr's recent book[1] there appears a table (p. 124) which lists several geographically restricted animal groups and indicates the percentage of species of each group that are mono-

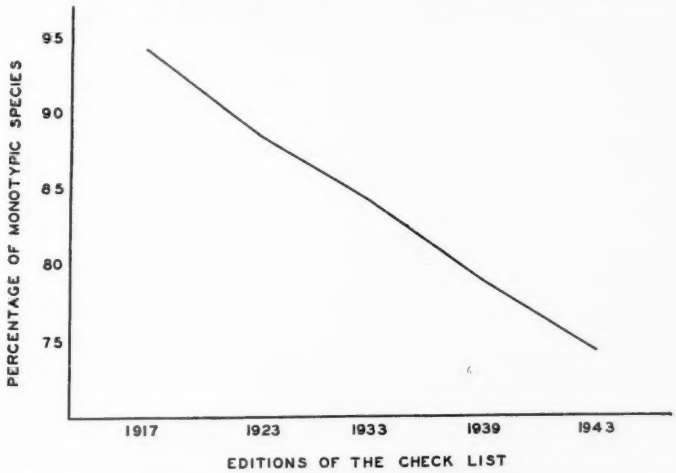

FIG. 1. Graph showing the percentage of monotypic species in succeeding editions of the "Check List of North American Amphibians and Reptiles."

[1] Ernst Mayr, "Systematics and the Origin of Species." New York, N. Y.: Columbia University Press. Pp. 1–334. 1942.

typic. Dr. Mayr suggests two conclusions to be drawn from this tabulation, the second of which is that "the percentage of monotypic species recorded in a certain group at a given period depends to a considerable extent on the degree of finality which the classification of the respective group has reached." Of the series of groups in his table, two pairs are pertinent in this regard. Miller listed 75.8 per cent. of the species of European mammals as monotypic in 1912, while Ökland regarded only 54.7 per cent. as monotypic in 1937. Hartert considered 37.7 per cent. of the species of Palearctic Passeres monotypic in 1923 and in 1936 reduced the percentage to 29.7.

The "Check List of North American Amphibians and Reptiles," which has been organized through five editions (1917, 1923, 1933, 1939 and 1943) by Leonhard Stejneger and Thomas Barbour, seemed to us to provide ideal material for a tabulation of this nature because it covers the same animal groups within the same geographic area and has been brought together by the same authors several times through a period of years. Fig. 1 summarizes the results of our census of these editions; it includes all the forms listed in each Check List except those that have been introduced into the area.

As the percentage of monotypic species of amphibians and reptiles was reduced from 94.2 to 74.4 the number of genera remained relatively stable (extremes being 121 and 132); the number of species increased slightly from 438 to 500; and the number of subspecies increased sharply from 497 to 751. The average number of forms (species and subspecies) per genus has increased regularly through the five editions from 4.1 in the first edition to 5.7 in the fifth edition and the average number of subspecies per species has increased regularly through the same period from 1.13 to 1.50.

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